

## Occurrence of diapause in *Orius laevigatus*

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### Abstract

One of the main problems in biological control of thrips in the Mediterranean area is that *Frankliniella occidentalis* (Pergande) does not undergo diapause. Therefore, finding a non-diapausing species or strain of the genus *Orius* would be very useful for seasonal inoculative releases to control this species of thrips. Both the palearctic *O. majusculus* (Reuter) and the nearctic *O. insidiosus* (Say) show a reproductive diapause that is induced by photoperiod. No data were available about the occurrence of diapause in *O. laevigatus* (Fieber). The possibility of inducing a reproductive diapause in this palearctic species was therefore investigated in the laboratory using two strains: strain N collected in northern Italy (Po Valley; ca. 44° N latitude) and strain S collected in southern Italy (Sicily; ca. 37° N latitude). The influence of photoperiod on eggs at 18±1°C, RH=75±10% and at several light regimes varying between 16L:8D and 8L:16D (experiment 1) and between 13L:11D and 11L:13D (experiment 2) was studied. *O. laevigatus* were fed on *Ephestia kuehniella* (Zeller) frozen eggs.

Development time, adult emergence, sex ratio, pre-oviposition period, fecundity up to day 29 of adult life, and the presence of mature oocytes were recorded. Photoperiods of 11.5L:12.5D and 12L:12D induced a longer development time, a longer pre-oviposition period and a lower oviposition rate than the other photoperiods for both populations. The percentage of egg-laying females at 18°C was higher for strain S (70%) than for strain N (44%). Termination of diapause was investigated by exposing the *Orius* strains to an higher temperature (26°C) and a longer day-length (16L:8D). The females of both strains supposedly in diapause, rapidly started to lay a high amount of eggs independently from the environmental conditions to which they were previously exposed. Next, the two strains of *O. laevigatus* were reared at five temperature regimes (24°C/12.5°C; 26°C/15°C; 21.5°C/6°C; 22°C/12.5°C; 18°C constant) that matched the photoperiod which induced the lowest oviposition (11.5L:12.5D) in the previous experiments. The longest development time was found for both strains at 26.5°C/6°C and the shortest at 26°C/15°C. A constant temperature of 18°C induced a slightly shorter development than the thermoperiod of 26.5°C/6°C in both populations. The lowest fecundity was recorded at 26.5°C/6°C and at 18°C constant for both strains, and 26°C/15°C induced the highest fecundity in the females of strain N. When the females were moved from thermoperiods of 18°C to 26°C and 16L:8D, oviposition did increase, and more than 80% of females of both strains laid eggs. In all the experiments the two strains of *O. laevigatus* gave different results.

Wild populations of *O. laevigatus* were collected in the field in August-November in Sicily and in the Po Valley and maintained in cages in the field in northern Italy (44° latitude N). During the winter, once a month females were taken from the field cages and put into a climatic chamber at 26±1°C, RH 75±10%. A high percentage of females laid eggs, particularly those of the Sicilian population.

In conclusion, the two strains of *O. laevigatus* have a different way to overwinter: in the northern strain part of the population undergoes a weak reproductive diapause, while for the southern strain overwintering can be better described as quiescence.

**Key words:** *Orius laevigatus*, strain, diapause, quiescence, photoperiod, thermoperiod, rearing, Italy, Po Valley, Sicily.

### Introduction

An important feature of insect life is their behavioural adaptation to the ubiquitous, seasonally changing environment. In many regions of the world, the biological conditions suitable for growth, development, and reproduction generally prevail only during part of the year. Many authors have studied diapause as a biological phase which occurs in many arthropods in order to survive when unfavourable seasonal conditions are present, even though not all forms of seasonal adaptations are associated with diapause (De Wilde, 1956; Mansingh, 1971; Hodek, 1973; Beck, 1980; Tauber *et al.*, 1986; Danks, 1987; Leather *et al.*, 1993). In general, the arrestment in development that enables living organisms to synchronise their life cycle with favourable environmental conditions and that avoids unfavourable conditions is called dormancy, and it can occur during all seasons. Two types of dormancy are usually distinguished in insects: quiescence and diapause. But intermediate conditions are also found and dormancy does not neces-

sarily mean diapause.

Quiescence is a reversible state, characterised by a reduction in metabolism as a direct response to exposure to environmental extremes, such as temperature or photoperiod, and which ends immediately when favourable conditions resume. Diapause is an active response of individuals resulting in a dynamic state of low metabolic activity for adaptation to seasonal cycles, so to predictable conditions. It can be divided into three phases: diapause induction or pre-diapause (in the sensitive stage of the insect), diapause maintenance (responsive stage) and diapause termination or post-diapause. The term 'diapause syndrome', coined by De Wilde (1959), is a general term for the species-specific set of behavioural and physiological symptoms of diapause, referring initially to pre-diapause preparation for the future seasonal conditions. This concept of 'diapause syndrome' has recently been enlarged to include all pre-diapause, diapause, and post-diapause processes to seasonal changes (Tauber *et al.*, 1986). Diapause is a physiologically dynamic developmental

stage and it occurs during genetically determined stage(s) of metamorphosis which are species-specific. Many factors (biotic and abiotic) can function as the token stimulus to induce diapause. In fact, the insects can translate the token stimuli in neurohormonal changes which lead to diapause (Williams, 1952). Often, the most common and reliable token stimulus is photoperiod. In many cases photoperiod and temperature interact, although other environmental factors such as food and water may also interfere (Beck, 1980; Saunders, 1982; Tauber *et al.*, 1986; Gullan and Cranston, 1994).

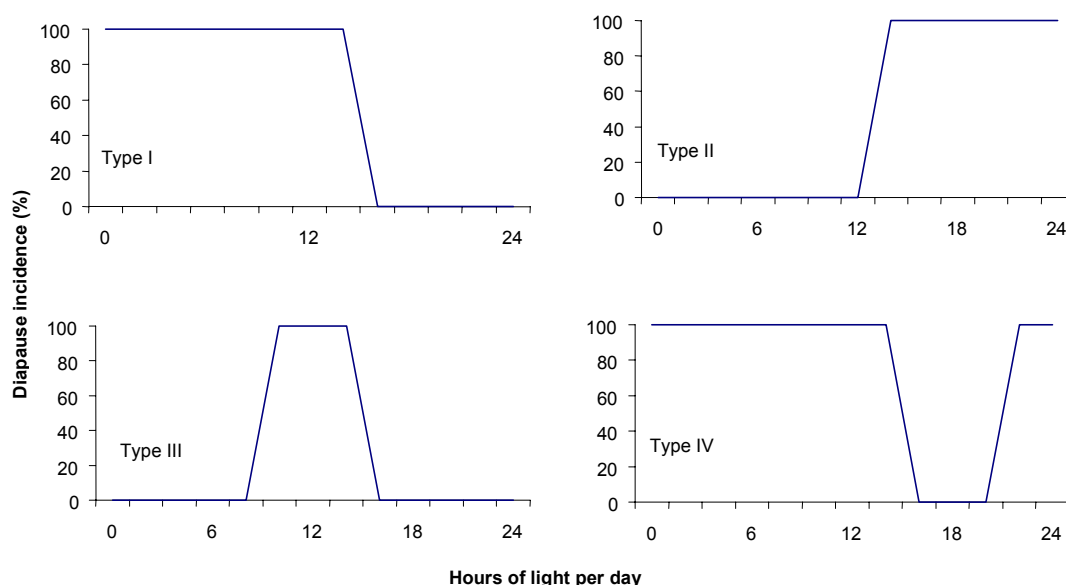
Beck (1980) defined four types of diapause response curves based on the photoperiod effects (figure 1). Type I is the long-day type of response which is typical of insects that reproduce, grow and develop under the long day conditions. Such insects go into diapause after experiencing the short days of late Summer and Autumn. Type II, the short-day type of response, is less common and is characteristic of insects that grow and reproduce under short day-length and that undergo aestival diapause. Type III is the photoperiodic response of species with both long-day and short-day responses showing two well-defined critical day-lengths. Type IV is demonstrated in only a few species, and is characterised by the absence of diapause incidence over a very restricted range of relatively long day-lengths. All other photoperiodic conditions result in a high incidence of diapause. This type of response might be termed a long-day-short-day response.

During the course of diapause, there is generally a decrease in diapause intensity and these changes can occur even if the insects are held under constant conditions. Photoperiod is one of the major factors which acts to

maintain diapause. Even the diapause termination can be dependent upon outside stimuli the insect receives, which generally are demonstrated by the return of suitable environmental conditions, such as light and temperature. Diapause intensity is generally inversely proportional to biological characteristics such as oxygen consumption, development rate, and the pre-oviposition period (Beck, 1980; Hodek and Honek, 1970). An indication of the intensity of diapause can be given by the difficulty of interrupting the diapause itself.

It is often not easy to describe the diapause syndrome of an insect species. According to many authors (*e.g.* Tauber *et al.*, 1986; Danks, 1987) there is no single 'correct' classification for diapause, as there is a series of cases in a continuum and adaptive responses to a variety of circumstances. A synthesis of the main diapause descriptions is shown in table 1. The classification of diapause most used in Europe is still that of Müller (1970), based chiefly on the concept of the intensity of dormancy as related to climate and geography.

Few data are available on the diapause syndrome in the predatory Heteroptera. Heteropteran predators from temperate climates generally show a seasonal activity typical of many insect species: they appear in spring or early summer and disappear in autumn (Ruberson *et al.*, 1998). All the Anthocoridae species studied overwinter as adults and those which undergo diapause in very different overwintering sites (leaf litter, organic material in wooded areas, in winter grasses or under tree bark) show reproductive diapause. Overwintering in the adult stage may provide the greatest flexibility for location of, and movement within overwintering sites, as well as movement towards food and reproductive resources in the spring.



**Figure 1.** Different types of diapause incidence-day-length relationship observed among insects (modified from Beck, 1980).

**Table 1.** Summary of terminology concerning diapause and quiescence.

Terminology	Main features	Induced by	Terminated	Author
D i a p a u s e				
Parapause	no clear induction phase	genetically fixed for univoltine species	Genetically	Müller, 1970
Diapause	long preparation before adverse situation	extreme and long-term adversity ( <i>i.e.</i> photoperiod)	—	Mansingh, 1971
	deep and continuous diapause	extreme and long-term adversity ( <i>i.e.</i> photoperiod)	—	Ushatnskaya, 1976
Obligatory diapause	present in every individual in each generation	regardless environmental conditions	—	Beck, 1980
Univoltine diapause	present in every individual in each generation	regardless environmental conditions	—	Tauber <i>et al.</i> , 1986
I n t e r m e d i a t e f o r m s o f d i a p a u s e				
Eudiapause	clear induction phase	facultative and due to unfavourable condition (usually photoperiod)	a different factor than the induction stimulus (for example temperature)	Müller, 1970
Oligopausa	facultative appears and ends with a delay relative to unfavourable conditions	unfavourable environmental conditions	end of unfavourable conditions	Müller, 1970
	short preparation before adversity	mild and long-term adversity	end of unfavourable conditions	Mansingh, 1971
	intermediate between quiescence and diapause	unfavourable environmental conditions	end of unfavourable conditions	Ushatnskaya, 1976
Facultative diapause	on an irregular basis in response to unpredictable exigencies	unfavourable environmental conditions	end of unfavourable conditions	Beck, 1980
Multivoltine diapause	on an irregular basis in response to unpredictable exigencies	unfavourable environmental conditions	end of unfavourable conditions	Tauber <i>et al.</i> , 1986
Q u i e s c e n c e				
Quiescence	immediate facultative retardation or stop of development	unfavourable conditions	end of unfavourable conditions	Müller, 1970

Some *Orius* spp., such as the nearctic species *O. insidiosus* (Iglinsky and Rainwater, 1950; Kingsley and Harrington, 1982; Ruberson *et al.*, 1991; van den Meiracker, 1994) and *O. tristicolor* (White) (Anderson, 1962; Askari and Stern, 1972; Gillespie and Quiring, 1993; van den Meiracker, 1994), as well as the palearctic species *O. majusculus* (Fischer *et al.*, 1992; van den Meiracker, 1994) undergo reproductive diapause under photoperiodic stimuli (type I of diapause induction, see figure 1). The palearctic *O. albidipennis* (Reuter) collected on the Canary Islands does not undergo reproductive diapause at photoperiods varying from 8:16 to 16:8 (L:D) (van den Meiracker, 1994). The palearctic species *O. niger* Wolff is known to overwinter (Bailov, 1929), even though van de Veire and Degheele (1992) found that this species is not affected by short day-length in contrast with *O. insidiosus*, but no specific studies were carried out with this predator. Péricart (1972) recorded that the palearctic *O. laevigatus* overwinters as an adult, but no data are available about the existence of a reproductive diapause in this species. However, Rudolf *et al.* (1993) wrote that *O. laevigatus* appears to show quiescence and not diapause, because when the individuals collected during the winter were put at favourable climatic conditions, they immediately started to lay eggs.

Differences in the response to overwintering cues among insects of the same species from different geographical areas have been found and critical photoperiods for diapause induction often appear related to latitude (Tauber *et al.*, 1986; Leather *et al.*, 1993). Two different populations of *O. tristicolor* undergo diapause at different critical photoperiods according to their different geographical distribution in USA (Gillespie and Quiring, 1993). Parker (1975) showed genetically controlled differences in the diapause induction of two populations of another species belonging to the family Anthoridae, *Anthocoris nemorum* (L.), collected in Scotland (56° N) and in southern England (51° N).

One of the main problems in the biological control of thrips is the synchronisation between prey and predators. In a large part of the southern Mediterranean area where temperature rarely decreases below 5–6°C during winter, *F. occidentalis* remains active in winter (Del Bene and Gargani, 1989; Lacasa, 1990; Marullo, 1991). Non-diapausing natural enemies are, of course, more suitable for control of thrips by seasonal inoculative releases during short-day-length periods in winter. Brødsgaard (1994) studied the influence of photoperiod on *F. occidentalis* and found that this thrips species showed only slight differences in development time, longevity, and fecundity when exposed to short vs long day-length.

The choice of methods for analysing the sensitive stage of an insect depends on the stage that enters diapause. In some species, both the sensitive stage and diapause stage occur in the same stage, but more frequently they are distinct (Tauber *et al.*, 1986). When the sensitive stage that undergoes diapause is the adult, this kind of dormancy is usually called reproductive diapause: the key arrestment in development in adults takes place in the ovaries (Beck, 19880; Saunders, 1982; Danks, 19887). From the literature it is known that the *Orius* species that show diapause, show a reproductive diapause (Péricart, 1972). The sensitive stage(s) of the Heteropterans that overwinter in diapause as adults, can comprise a large segment of the insect's lifespan (Ruberson *et al.*, 1998). Generally, the lack of juvenile hormone induces diapause in the adults (Tauber *et al.*, 1986). *O. sauteri* (Poppius) was found to undergo diapause at a short day-length (between 13 and 14 hours of light) and the sensitive stage here was the nymph (personal communication, E. Yano, 1996). In many insects that have a reproductive diapause, the sensitive stages are the last instars, e.g. *A. nemorum* and *Chrysoperla carnea* (Stephens) (Danks, 1987).

The aim of this study was to investigate the effect of photoperiod, as well as its interaction with temperature, on the life cycle of two strains of *O. laevigatus*, in order to determine the existence of diapause.

## Materials and Methods

The sensitivity of *O. laevigatus* to photoperiod and temperature was tested in three laboratory and one field-laboratory experiment using two strains: strain N [northern strain, collected in northern Italy at ca. 44° N latitude (Po Valley)] and, strain S [southern strain, collected in southern Italy at ca. 37° N latitude (Sicily)]. For the first three laboratory experiments, both strains were reared in the laboratory for ca. 12 generations starting from a few hundred individuals per strain. They were fed with *Ephestia kuehniella* (Zeller) frozen eggs and could oviposit on bean pods (*Phaseolus vulgaris* L.). During all four experiments predators were fed *ad libitum* on *E. kuehniella* frozen eggs glued onto cardboard. Water was supplied by wet cotton. In the first three experiments sex ratio was determined at adult emergence. Newly-emerged adults were kept in groups during five days for mating. Pairs were then isolated into cylindrical cages (4 cm high and 4 cm diameter) and supplied with a piece of bean pod for oviposition. Dead males were regularly removed and replaced with living ones.

During all the experiments, random samples of *O. laevigatus* eggs ( $n > 100$ /exposure/strain) were checked for hatching. The bean pods were kept in glass tubes at 26°C (16L:8D) and hatching was checked after one week by counting the open opercules under a stereomicroscope.

At the end of each experiment, the surviving females were maintained for 24 hours without bean pods, then they were killed and dissected in order to count the number of mature oocytes. The relationship between

fecundity and the number of mature oocytes per female was investigated in all experiments apart from test 1 in experiment 1.

## Influence of photoperiod

### Experiment 1

Induction of diapause by photoperiod was tested at a fixed temperature of 18±1°C, at a light intensity of ca. 1,800 lx and RH = 75±10%. Bean pods with 0-6 h old *O. laevigatus* eggs were put into 5 incubators set at different photoperiods: 8L:16D, 10L:14D, 12L:12D, 14L:10D, and 16L:8D (number of eggs > 800/photoperiod). Eighteen degrees Celsius was chosen because it is the mean temperature in Italy during October-November in the Po Valley, and November-December in southern Italy, when potential diapause induction of *O. laevigatus* may occur. Furthermore, 18°C is the mean temperature recorded in the northern European greenhouses early in the season (van den Meiracker, 1994a). The incubators were placed in a dark chamber to prevent light interference during checks. After hatching, groups of nymphs were kept in transparent cylindrical plexiglass cages (9 cm high and 9 cm diameter) covered with gauze for aeration until adult emergence. During 24 days after isolation of adults in pairs, mortality and fecundity were checked. A period of 24 days is about 7 days longer than the estimated pre-ovipositional period of *O. laevigatus* reared at 18°C and 16L:8D (Alauzet *et al.*, 1994) and 8 times longer than the pre-oviposition period recorded at 26°C which is the optimal temperature for *O. laevigatus* (Tommasini, 2003). Females which did not lay eggs within 29 days after emergence were excluded from the calculation of the pre-oviposition period.

In test 1 of experiment 1, only strain N was considered, and hatching and adult emergence was checked every 4 hours. The number of pairs tested ranged from 47 to 72 per photoperiod and oviposition was determined daily. In test 2 of experiment 1, both strain N and strain S were considered and adult emergence was checked twice a day. The number of pairs tested ranged from 28 to 44 per photoperiod and oviposition was recorded three times a week.

### Experiment 2

Five intermediate day-lengths were set up (13L:11D; 12.5L:11.5D; 12L:12D; 11.5L:12.5D; 11L:13D) based on the results obtained during experiment 1 and close to the natural photoperiod in autumn and spring. The same procedure adopted in test 2 of experiment 1 was followed. The development time ( $n = 500$  eggs) per photoperiod and the pre-oviposition period were checked daily and every two days respectively. Females which did not lay eggs within 29 days after emergence were excluded from the calculation of the pre-oviposition period. For each photoperiod survival and fecundity were checked three times a week for 24 days after isolation of pairs (44 to 50 pairs per photoperiod). After this period, pairs were moved to another climatic chamber at 26±1°C and photoperiod 16L:8D where the same checks were continued for 24 additional days.

**Table 2.** Correspondence of thermoperiods with the seasonal periods in the Po Valley and Sicily.

Thermoperiod (°C)	Correspondence with the seasonal period (from - to)	Mean temperature (°C)
24 / 12.5	Po Valley: Sept. 20 - Oct. 10	18
26 / 15	Sicily: Oct. 1 - Oct. 20	20
21.5 / 6	Po Valley: Oct. 1 - Oct. 20	16.5
22 / 12.5	Sicily: Oct. 20 - Nov. 20	17
18 constant	Po Valley: Sep. - Oct. Sicily: Nov. - Dec.	18

### Influence of temperature

#### Experiment 3

Five temperature regimes were tested (24/12.5°C; 26/15°C; 21.5/6°C; 22/12.5°C; 18°C constant) that matched the photoperiods that induced the lowest oviposition in both strains in the previous experiments (11.5L:12.5D). The higher temperatures of the thermoperiods coincided with the photophase and the lower temperatures with the scotophase. The autumn temperatures of five years (1989-1992) and two years (1992 and 1993) recorded at several meteorological cabins placed respectively in the Po Valley (ca. 44°N) and on Sicily (ca. 37°N), were analysed to choose the thermoperiods for the experiment (table 2).

The same procedure as in test 2 of experiment 1 was followed and more than 500 eggs per thermoperiod were used for testing. The development time and pre-oviposition period were recorded. Females which did not lay eggs within 29 days after emergence were excluded from the calculation of the pre-oviposition period. Forty-eight to 51 pairs per temperature regime were isolated five days after emergence. Female survival and fecundity were determined during 24 days after pairs were isolated, and the pairs were observed for an additional 24 days after being transferred to 26°C and 16L:8D.

### Incidence of diapause in field collected populations

#### Experiment 4

The egg-laying tendency of *O. laevigatus* females collected in nature during autumn and winter at two different latitudes were measured. Wild populations of *O. laevigatus* were collected in autumn on Sicily (August-

November 1994) and in the Po Valley (August-October 1994). The adults collected were phenotypically identified (Tommasini, in press) and maintained in glass jars put in a meteorological cabin in open air in northern Italy (ca. 44° N), with possibility to feed on *E. kuehniella* frozen eggs and with bean pods for oviposition. Absorbent paper was added as shelter and to prevent excessive humidity. New bean pods and prey were provided weekly. From August 1994 up to February 1995 some females were isolated every month in plexiglass cylinders (4 cm high, 4 cm diameter) with *E. kuehniella* frozen eggs and a piece of bean pod for oviposition in a climatic chamber at 26±1°C, RH=75±10% and photoperiod 16L:8D. Thirteen to 55 females per month per population were observed with the exclusion of September for strain S and February for strain N because an insufficient number of females was found in the field. The beginning of oviposition of each female was recorded three times a week for a maximum period of three weeks.

### Statistical Analysis

For each condition tested in the different experiments, development time (from egg to adult emergence), pre-oviposition period, percentage of ovipositing females, fecundity, and fertility of eggs, data were statistically analysed. To obtain normal distributions, data were log transformed before analysis when necessary.

#### Experiment 1

Pre-imaginal development times, pre-oviposition times and female longevity during the experiment, were compared using one-way analysis of variance (ANOVA) and Tukey's test ( $P < 0.05$ ). Total oviposition was compared using the Kruskal-Wallis test followed by Dunn's procedure for multiple comparison. Percentage of emerged adults as well as the sex ratio, the percentage of egg-laying females, the percentage of females (ovipositing and non-ovipositing) with and without mature oocytes and percentages of surviving females at the end of the experiment were compared with  $\chi^2$  test ( $P < 0.05$ ). A linear correlation was established between the number of mature oocytes in the female's abdomen at day 30 and the total oviposition during the initial 29 days of adult life.

**Table 3.** Development time, pre-imaginal mortality and sex ratio of *O. laevigatus* (strain N) reared at 18°C and at five photoperiods (experiment 1, test 1). Same letters indicate no significant differences by ANOVA and Tukey's test ( $P < 0.05$ ) (Means ± SE).

Photoperiod (L:D)	Embryonic development (days)	No. of nymphs tested	Post-embryonic development (days)	Total development (days)	% Pre-imaginal mortality	Sex ratio (% females)
8:16	8.2 ± 0.1 a	462	22.7 ± 0.4 a	30.9 ± 0.3 a	51.1	43.4
10:14	8.1 ± 0.1 a	375	23.7 ± 0.3 a	31.8 ± 0.3 a	59.2	51.6
12:12	9.2 ± 0.1 b	449	28.5 ± 0.2 c	37.7 ± 0.2 c	64.4	45.6
14:10	9.6 ± 0.1 b	326	28.2 ± 0.3 c	37.8 ± 0.3 c	69.9	58.2
16:8	8.5 ± 0.2 a	476	25.6 ± 0.5 b	34.1 ± 0.3 b	60.7	47.6

## Experiment 2 and 3

Development time and fecundity were analysed by one-way analysis of variance (ANOVA), as well as for pre-oviposition time and females longevity during the experiment. When significant differences were found with ANOVA, means were separated using Tukey's test ( $P < 0.05$ ). A covariance analysis (ANCOVA) was carried out for both strains on the total fecundity (exposure 1+2), considering the fecundity during the first 29 days after emergence at different photoperiods or thermoperiods (exposure 1) as covariate. When differences were found, Tukey's test was performed ( $P < 0.05$ ). The percentages of hatching, of pre-imaginal mortality, the sex ratio as well as the percentages of egg-laying females, of surviving females and of fertile and infertile females with and without oocytes were compared with  $\chi^2$  test ( $P < 0.05$ ). A correlation between total fecundity and mature oocytes in the females abdomen at day 54 after emergence was calculated assuming  $y = a + bx$ .

## Experiment 4

The percentages of the females that laid eggs after three days and three weeks was compared with  $\chi^2$  test ( $P < 0.05$ ).

## Results

### Influence of photoperiod

#### Experiment 1

##### Test 1:

Table 3 reports the pre-imaginal development times of strain N of *O. laevigatus* exposed to different photoperiods. No differences were found in the sex ratio of emerged adults, so all the data were considered together. The longest development times were recorded at 12L:12D and 14L:10D. The shortest development times were recorded at the shortest day-lengths (8L:16D and 10L:14D). Differences in pre-imaginal mortality were found among all the photoperiods tested ( $\chi^2$ ,  $P < 0.05$ ). The highest mortality was recorded at 14L:10D, while the lowest was at the shortest day-length. No differences among the pre-oviposition period were recorded by ANOVA, thus the photoperiod did not seem to influence strain N (table 4). Significant differences were re-

corded in the number of eggs laid per female between photoperiods 12L:12D and 16L:8D (table 5), as well as in the percentage of egg-laying females, where the lowest percentages were found at photoperiods 12L:12D ( $\chi^2$ ,  $P < 0.05$ ) (table 4). However, none of the photoperiods tested induced diapause in all females. At the photoperiods 16L:8D, 14L:10D and 12L:12D significant differences in the percentage of females with mature oocytes were found at the end of the experiment ( $\chi^2$ ,  $P < 0.05$ ) (table 5). Figure 2 indicates that the intermediate photoperiod induced a low oviposition activity of *O. laevigatus*. At the end of the experiment no differences were recorded in numbers of surviving females among photoperiods ( $\chi^2$ ,  $P > 0.05$ ).

**Table 4.** Pre-oviposition period and percentage of egg-laying females of *O. laevigatus* (strain N) during experiment 1, test 1 (18°C and five photoperiods). No significant differences were recorded by ANOVA ( $P > 0.05$ ) (Means  $\pm$  SD).

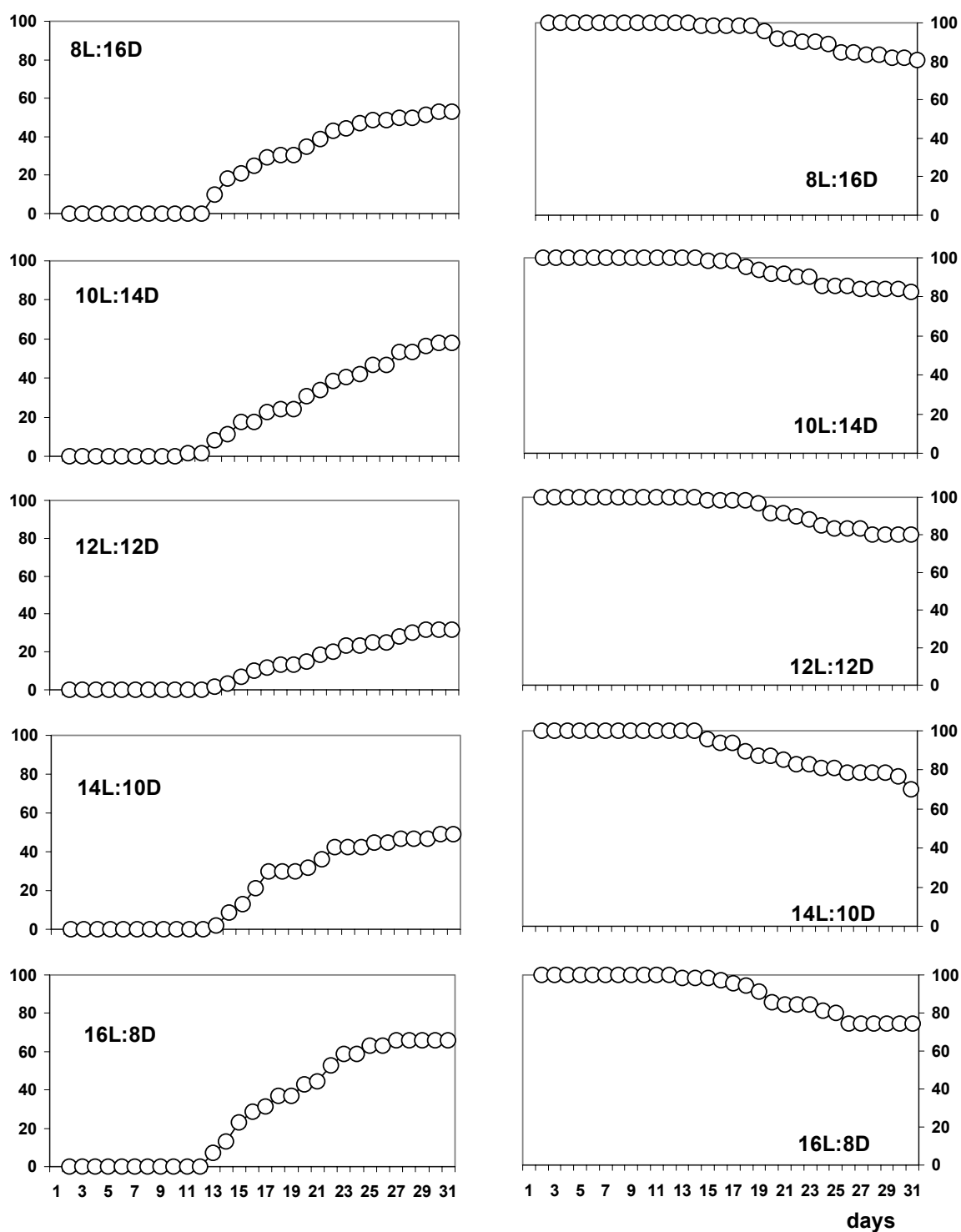
Photoperiod (L:D)	No. of pairs	Pre-oviposition period (days)	% Egg-laying females
8:16	38	11.1 $\pm$ 4.9	52.8
10:14	36	13.2 $\pm$ 5.8	58.1
12:12	19	13.3 $\pm$ 5.1	31.7
14:10	24	11.5 $\pm$ 4.4	48.9
16:8	46	11.3 $\pm$ 4.1	65.7

**Table 5.** Fecundity of females and percentage of females with mature oocytes of *O. laevigatus* (strain N) during experiment 1, test 1 (18°C and five photoperiods). Same letters indicate no significant differences by Kruskal-Wallis test (Means  $\pm$  SD) ( $P < 0.05$ ).

Photoperiod (L:D)	No. of pairs	No. of eggs/female	% females with mature oocytes
8:16	72	16.2 $\pm$ 23.1 ab	56.1
10:14	62	13.1 $\pm$ 19.3 ab	59.3
12:12	60	5.9 $\pm$ 12.9 a	43.6
14:10	47	15.1 $\pm$ 20.8 ab	68.9
16:8	70	21.6 $\pm$ 23.3 b	75.8

**Table 6.** Pre-imaginal development (egg-adult), pre-imaginal mortality and sex ratio of *O. laevigatus* (strains S and N) at different photoperiods (experiment 1 test 2). Same letters indicate no significant differences by ANOVA and Tukey's test ( $P < 0.05$ ) (Means  $\pm$  SE).

Photoperiod (L:D)	Strain N			Strain S		
	Total development (days)	% Pre-imaginal mortality	Sex ratio (% female)	Total development (days)	% Pre-imaginal mortality	Sex ratio (% female)
8:16	34.3 $\pm$ 0.4 a	82.3	60.4	34.7 $\pm$ 0.3 a	80.5	55.2
10:14	37.6 $\pm$ 0.4 b	83.9	61.8	37.1 $\pm$ 0.3 bc	85.3	57.3
12:12	41.7 $\pm$ 0.4 c	87.9	61.5	38.8 $\pm$ 0.4 d	85.0	55.8
14:10	37.5 $\pm$ 0.3 b	85.6	49.4	36.2 $\pm$ 0.4 b	74.2	51.9
16:8	34.2 $\pm$ 0.3 a	77.3	54.1	38.3 $\pm$ 0.5 cd	85.2	59.5



**Figure 2.** Cumulative percentage of egg-laying (on the left) and surviving (on the right) females of *O. laevigatus* (strain N) reared at different photoperiods and 18°C (Experiment 1, test 1).

The percentage of females with mature oocytes at day 30 from adult emergence shows significant differences ( $\chi^2$  test,  $P < 0.05$ ) among photoperiods. The highest percentage was observed at 16L:8D and the lowest one at 12L:12D, although it did not differ from that recorded at photoperiods 8L:16D and 10L:14D.

#### Experiment 1

##### Test 2:

The total development times of both strains of *O. laevigatus* are reported in table 6. No differences were found in the sex ratio of emerged adults, so both sexes were considered together for development time ( $\chi^2$ ,

$P > 0.05$ ). The longest development times were recorded at 12L:12D for both strains and 16L:8D for strain S only. Intermediate development times were recorded at 10L:14D and 14L:10D for both strains. Compared to test 1, a higher pre-imaginal mortality at all photoperiod regimes was observed and no differences were recorded among strains and regimes by the  $\chi^2$  test ( $P > 0.05$ ).

The pre-oviposition periods showed neither differences at the five photoperiod regimes (ANOVA,  $P = 0.07$ ), nor between strains and the interaction of strains and photoperiods. This result is strongly influ-

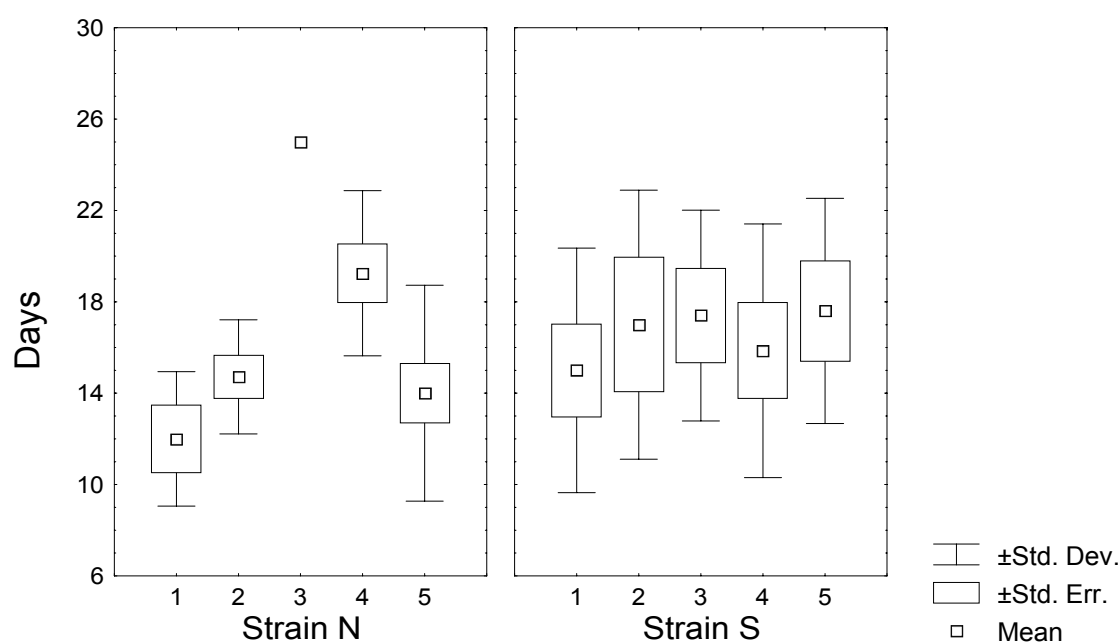
enced by the limited data recorded for strain N at the photoperiod 12L:12D (tables 7 and 8). Only one female laid eggs during exposure 1 at photoperiod 12L:12D. Therefore, to detect a possible difference, another ANOVA was carried out and all the females (egg-laying and not) were considered. For no-egg-laying females, the pre-oviposition time was taken at 29 days (the period of the experiment) (table 7). Now, a difference was recorded among regimes. Photoperiod 12L:12D induced the longest pre-oviposition period in strain N of *O. laevigatus* (figure 3).

**Table 7.** ANOVA summary of the main effects on the pre-oviposition period of *O. laevigatus* (strains N) during the exposure at five photoperiods and 18°C (experiment 1, test 1) considering only egg-laying females, and all the females respectively.

Effect		Only egg-laying females			All females	
		df	F	P-level	F	P-level
Strain	(1)	1	0.08	0.77	0.78	0.37
Regimes	(2)	4	2.25	0.07	2.80	0.02*
Interaction	(1 x 2)	4	2.03	0.10	1.98	0.09

**Table 8.** Pre-oviposition period of *O. laevigatus* (strains S and N) in experiment 1, test 2 (18°C and different photoperiods). No significant differences were found by ANOVA ( $P > 0.05$ ) (Means  $\pm$  SD). Percentage of egg-laying females of *O. laevigatus* of the entire population exposed to different photoperiods ( $\chi^2$  test;  $P < 0.05$ ).

Photoperiod (L:D)	No. of pairs	Strain N		No. of pairs	Strain S	
		Pre-oviposition period (days)	% Egg-laying females		Pre-oviposition Period (days)	% Egg-laying females
8:16	4	12.0 $\pm$ 2.9	11.1	7	15.0 $\pm$ 5.4	15.9
10:14	7	14.7 $\pm$ 2.5	20.6	3	17.0 $\pm$ 5.9	12.1
12:12	1	25.0 $\pm$ 0.0	3.5	5	17.4 $\pm$ 4.6	13.2
14:10	8	19.3 $\pm$ 3.6	28.6	7	15.9 $\pm$ 5.6	17.5
16:8	13	14.0 $\pm$ 4.7	39.4	5	17.6 $\pm$ 4.9	16.7



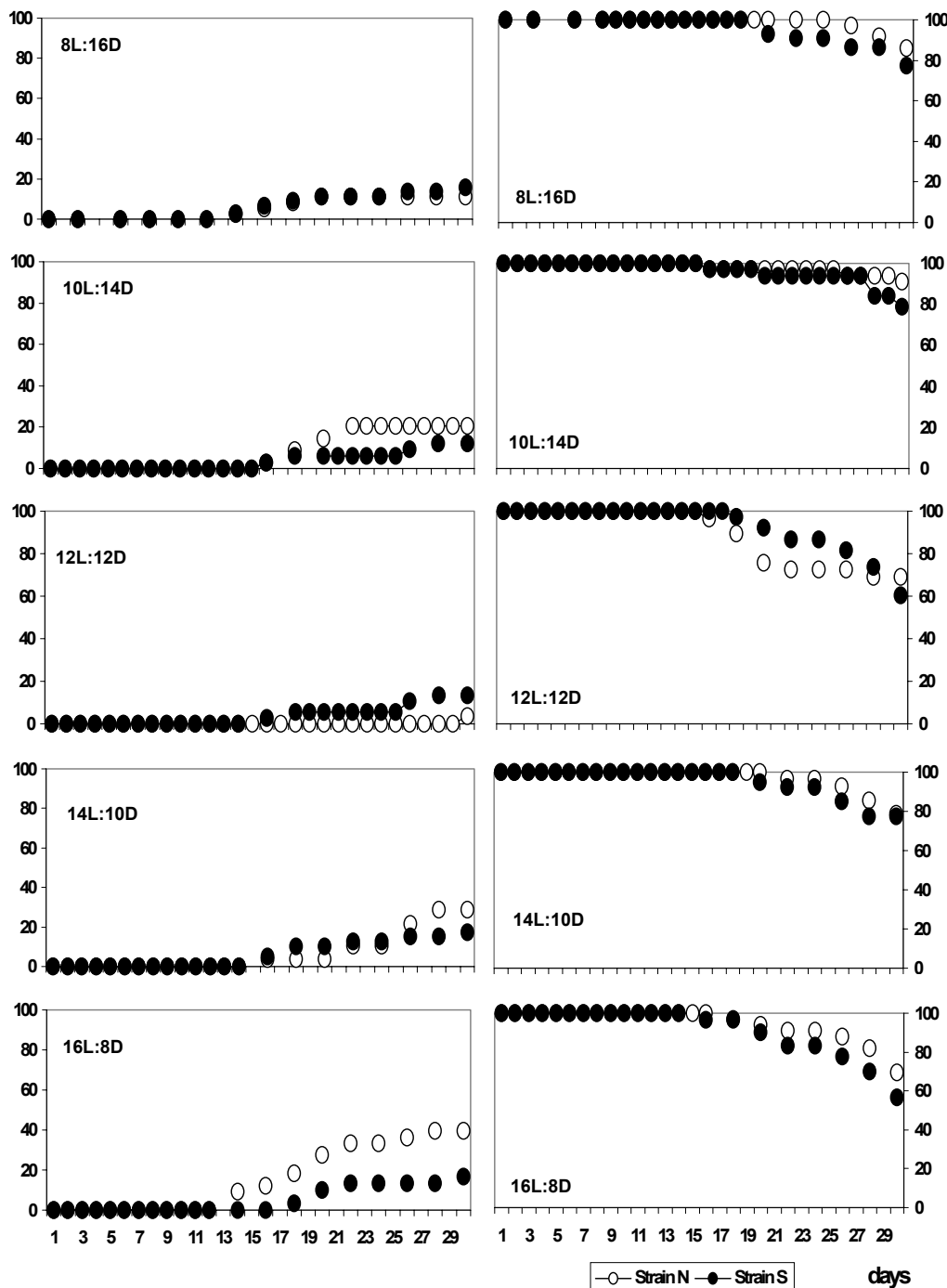
**Figure 3.** Pre-oviposition period of *O. laevigatus* (strains N and S) at different photoperiods (1, 8L:16D; 2, 10L:14D; 3, 12L:12D; 4, 14L:10D; 5, 16L:8D) and 18°C. (Experiment 1, test 2).



The percentage of egg-laying females is given in table 8, the number of eggs laid per female in table 9. An increase in the oviposition from day 15 to day 29 of adult life was observed (figure 4). Compared to test 1, strain N showed a lower oviposition rate, but the previous trend found for the oviposition activity was confirmed.

Presumably, the lower oviposition in test 2 is due to the reduction in the number of checks. The lower number of checks was done to keep low temperature (18°C) more constant. The number of eggs laid per female at 12L:12D was lower than that at 16L:8D, and

the percentage of egg-laying females was lower at 12L:12D and at 8L:16D compared to the other photoperiods tested. In strain N, only 1 female laid eggs 23 days after emergence at 12 hours photophase. Strain S showed no differences among groups exposed to different day-lengths, indicating a lower sensitivity to influence of photoperiod than strain N, although each group showed a lower percentage of egg-laying females compared to strain N reared at 16L:8D ( $\chi^2$  test;  $P < 0.05$ ). Almost all the eggs checked during experiment 1 were fertile (hatching rate >90%).



**Figure 4.** Cumulative percentage of egg-laying (on the left) and surviving (on the right) females of *O. laevigatus* (strains N and S) at different photoperiods and 18°C (Experiment 1, test 2).

**Table 9.** Fecundity of *O. laevigatus* females, during the initial 29 days of adult life at different photoperiods in experiment 1, test 2. Same letters indicate no significant differences by ANOVA and Tukey's test ( $P < 0.05$ ) (Means  $\pm$  SE).

Photoperiod (L:D)	Strain N		Strain S	
	No. of pairs	No. of eggs/female	No. of pairs	No. of eggs/female
8:16	36	$3.6 \pm 2.0$ ab	44	$4.0 \pm 1.7$ ab
10:14	34	$3.2 \pm 1.3$ ab	33	$2.5 \pm 1.6$ ab
12:12	29	$0.1 \pm 0.1$ a	38	$2.4 \pm 1.1$ ab
14:10	28	$5.2 \pm 1.8$ ab	40	$3.4 \pm 1.7$ ab
16:8	33	$11.2 \pm 3.4$ b	30	$2.7 \pm 1.7$ ab

**Table 10.** Percentage of fertile and infertile females with mature oocytes, and unfertile females without oocytes counted in the entire population, 24 hours after the end of test 2 of experiment 1 (day 30 after adult emergence).

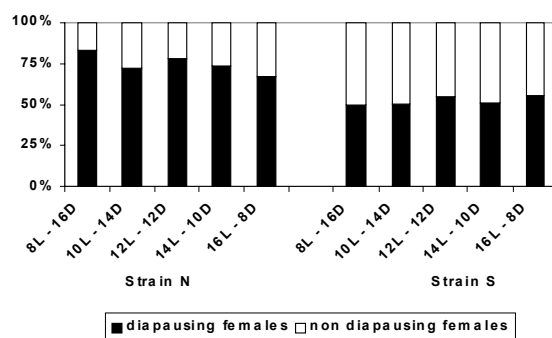
Photoperiod (L:D)	Strain N				Strain S			
	No. of females	% Fertile female with oocytes	% Infertile female with oocytes	% Infertile female without oocytes	No. of females	% Fertile female with oocytes	% Infertile female with oocytes	% Infertile female without oocytes
8:16	35	11.4	11.5	77.1	37	18.9	33.0	48.1
10:14	33	18.2	30.3	51.5	31	12.9	36.5	50.6
12:12	29	0	10.3	89.7	36	11.1	32.2	56.7
14:10	28	28.6	28.6	42.9	37	16.2	43.3	40.5
16:8	29	37.9	32.1	30.0	29	17.2	25.5	57.3
$\chi^2$ test		$P < 0.001$	$P > 0.05$	$P < 0.005$		$P > 0.05$	$P > 0.05$	$P > 0.05$

The cumulative percentages of surviving females of the two strains of *O. laevigatus* at the five photoperiods are shown in figure 4. No differences in survival were recorded among photoperiods for each strain, as well as between strains for each photoperiod ( $\chi^2$  test;  $P > 0.05$ ).

The percentages of females with mature oocytes at day 30 after adult emergence, the percentages of egg-laying females and the oviposition activities, showed a similar trend for the environmental conditions tested. However, strain S showed similar results among photoperiods and no statistical differences were found ( $\chi^2$  test;  $P > 0.05$ ) (table 10). In strain N significant differences were found in the percentage of fertile females with oocytes and among the percentage of infertile females without oocytes ( $\chi^2$  test;  $P < 0.05$ ) (table 10). The lowest percentage of infertile females without oocytes, supposedly in diapause, was found at photoperiod 16L:8D. The highest percentage was found at photoperiods 12L:12D and 8L:16D. The percentages of diapausing (= non-egg-laying) and non-diapausing (= egg-laying) females with oocytes in their abdomen at the end of experiment 1, test 2, are shown in figure 5. It appears that a high percentage of females of both strains of *O. laevigatus* and particularly of strain N, could be considered in diapause according to the just mentioned criteria. But we have to take into account that even at 16L:8D and 18°C more than 40% of the females did not lay eggs and did not produce oocytes. Thus, at the conditions that resulted in maximal diapause, the percentage of females not laying and not producing oocytes increased with 89.7% for strain N and with 57.3% for strain S.

A slight positive correlation was found between the number of mature oocytes in the abdomen of 30 days

old females (= 26 days after isolation of pairs) and the total number of eggs laid per female ( $R = 0.53$ ,  $P < 0.001$ ,  $y = 0.45 + 1.3x$ ).



**Figure 5.** Percentages of egg-laying (= non-diapausing) females and non-egg-laying (= diapausing) females with oocytes at the end of the experiment (Experiment 1, test 2) at different Light – Dark periods.

## Experiment 2

The development time of the two strains of *O. laevigatus* showed no differences when reared at the same photoperiod regime and both strains showed the longest development time at 11.5L:12.5D, 12L:12D and 13L:11D (tables 11 and 12). No differences were found in the sex ratio of emerged adults ( $\chi^2$  test), so all individuals were considered together. For the pre-oviposition period only a difference among the photoperiod regimes was recorded (table 13). Therefore Tukey's test was carried out among

the means pooled without distinguishing the strains. For both strains the pre-oviposition time was shorter at photoperiods 12.5L:11.5D and 13L:11D, when compared to that at photoperiod 11.5L:12.5D (tables 13, 14 and 15).

**Table 11.** Summary of the main effects found by ANOVA on the development time of *O. laevigatus* (strains N and S) during experiment 2.

Effect		df	F	P-level
Strain	(1)	1	4.02	0.06
Regimes	(2)	4	15.28	0.001 *
Interaction	(1 x 2)	4	1.07	0.41

During the initial 29 days of adult life at 18°C, the fecundity of the two strains of *O. laevigatus* showed differences related to both the strain and the photoperiod, as well as to the interaction of both photoperiod and strain (ANOVA,  $P < 0.01$ ) (table 13). No differences in the fecundity were recorded among the photoperiods for strain N, while strain S showed a higher rate of oviposi-

tion at 12.5L:11.5D compared to 11.5L:12.5D, 12L:12D, and the data recorded for strain N at all the photoperiods tested (table 16). During exposure 1, the percentage of egg-laying females increased progressively at all photoperiods and at the last day at this exposure, strain S showed a higher percentage of fertile females compared to strain N, respectively at photoperiods 11L:13D, 12.5L:11.5D and 13L:11D ( $\chi^2$  test,  $P < 0.001$ ) (table 15). However, no differences were recorded in the percentage of egg-laying females at the end of the experiment ( $\chi^2$  test,  $P > 0.05$ ) (table 19). The optimal climatic conditions during exposure 2, led to an increase in oviposition and in the percentage of fertile females (figure 7) for all groups of females, with no differences on total fecundity (exposure 1 + 2) among the five photoperiod regimes (ANCOVA,  $P > 0.05$ ) (table 19). Because the environmental conditions were changed in exposure 2 (26°C, 16L:8D), a covariance analysis was carried out and the fecundity during exposure 1 was taken as covariate. Significant differences were recorded only between strains and their interaction with the photoperiods (ANCOVA,  $P < 0.05$ ) (table 17).

**Table 12.** Development time (days) of two strains of *O. laevigatus* reared at 18°C and five photoperiods (experiment 2), starting from more than 500 eggs/strain/photoperiod. Different letters on the same line indicate a significant difference using Tukey's test on the means pooled ( $P < 0.05$ ) (Means  $\pm$  SD).

Photoperiod (L:D)	11:13	11.5:12.5	12:12	12.5:11.5	13:11
Strain N	34.5 $\pm$ 0.7	39.5 $\pm$ 0.7	36.5 $\pm$ 0.7	32.0 $\pm$ 2.6	35.7 $\pm$ 1.5
Strain S	34.0 $\pm$ 1.4	39.5 $\pm$ 0.7	39.5 $\pm$ 0.7	33.5 $\pm$ 0.7	38.0 $\pm$ 0.8
Means pooled	34.3 $\pm$ 1.0 ab	39.5 $\pm$ 0.7 c	38.0 $\pm$ 0.7 c	32.8 $\pm$ 1.6 a	36.9 $\pm$ 1.1 bc

**Table 13.** Summary of the main effects found by ANOVA on the pre-oviposition period and on the fecundity of *O. laevigatus* (strains N and S) when exposed to five photoperiods and 18°C (experiment 2).

Effect		Pre-oviposition period			Fecundity	
		df	F	P-level	F	P-level
Strain	(1)	1	2.24	0.13	25.72	0.001 *
Regimes	(2)	4	4.23	0.05*	5.84	0.001 *
Interaction	(1 x 2)	4	0.67	0.62	3.35	0.01 *

**Table 14.** Comparison of pooled means of the pre-oviposition period of the two strains of *O. laevigatus* (Tukey's test,  $P < 0.05$ ) (experiment 2).

Photoperiod (L:D)	11:13	11.5:12.5	12:12	12.5:11.5	13:11
Strains N and S	13.8 $\pm$ 4.5 ab	14.6 $\pm$ 6.0 b	14.2 $\pm$ 4.8 ab	11.5 $\pm$ 4.4 a	11.6 $\pm$ 5.2 a

**Table 15.** Pre-oviposition period and percentage of fertile females of two strains of *O. laevigatus* reared at 18°C and five photoperiods (experiment 2). Different letters indicate a significant difference using ANOVA and Tukey's test ( $P < 0.05$ ) (Means $\pm$ SD).

Photoperiod (L:D)	Strain N			Strain S		
	No. of fertile females	Pre-oviposition period (days)	% Fertile females	No. of fertile females	Pre-oviposition period (days)	% Fertile females
11:13	13	14.5 $\pm$ 5.0 ab	31.0	30	13.0 $\pm$ 4.0 ab	62.5
11.5:12.5	22	14.6 $\pm$ 6.1 b	52.4	22	14.6 $\pm$ 5.9 b	55.0
12:12	18	14.6 $\pm$ 4.7 ab	40.9	24	13.8 $\pm$ 5.0 ab	64.9
12.5:11.5	27	12.9 $\pm$ 4.5 a	61.4	42	10.2 $\pm$ 4.4 a	95.5
13:11	14	11.7 $\pm$ 5.9 a	34.1	29	11.6 $\pm$ 4.5 a	70.7

**Table 16.** Fecundity of two strains of *O. laevigatus* reared at 18°C and five photoperiods (experiment 2). Different letters indicate a significant difference using ANOVA and Tukey's test ( $P < 0.05$ ) (Means $\pm$ SD).

Photoperiod (L:D)	Strain N		Strain S	
	No. of pairs	No. of eggs/female	No. of pairs	No. of eggs/female
11:13	42	3.8 $\pm$ 7.7 a	48	11.5 $\pm$ 12.6 ab
11.5:12.5	42	7.3 $\pm$ 10.8 a	40	5.8 $\pm$ 7.0 a
12:12	44	3.9 $\pm$ 7.9 a	37	8.5 $\pm$ 10.1 a
12.5:11.5	44	8.3 $\pm$ 10.7 a	44	19.3 $\pm$ 16.0 b
13:11	41	3.3 $\pm$ 7.1 a	41	12.2 $\pm$ 13.7 ab

**Table 17.** Summary of the main effects found by ANCOVA on the fecundity of *O. laevigatus* (strains N and S) during experiment 2, considering fecundity during exposure 1 as covariate; the entire female population was included.

Effect	df	F	P-level
Strain (1)	1	12.10	0.001 *
Regimes (2)	4	1.39	0.2
Interaction (1 x 2)	4	2.85	0.02 *

The covariance analysis was carried out either considering the full population tested or only the egg-laying female population for both strains as shown in table 18. Similar differences were recorded for fecundity between *O. laevigatus* strains and their interaction with environmental conditions (regimes) (tables 17 and 18) and a test of parallelism confirmed those results. The main effects found by ANCOVA on the full populations are shown in figure 6, which gives a clear idea of the behaviour of the two strains of *O. laevigatus* at the different regimes tested.

**Table 18.** Summary of the main effects found by ANCOVA on the fecundity of *O. laevigatus* (strains N and S) during experiment 2, considering fecundity during exposure 1 as covariate; only fertile females of the populations were included.

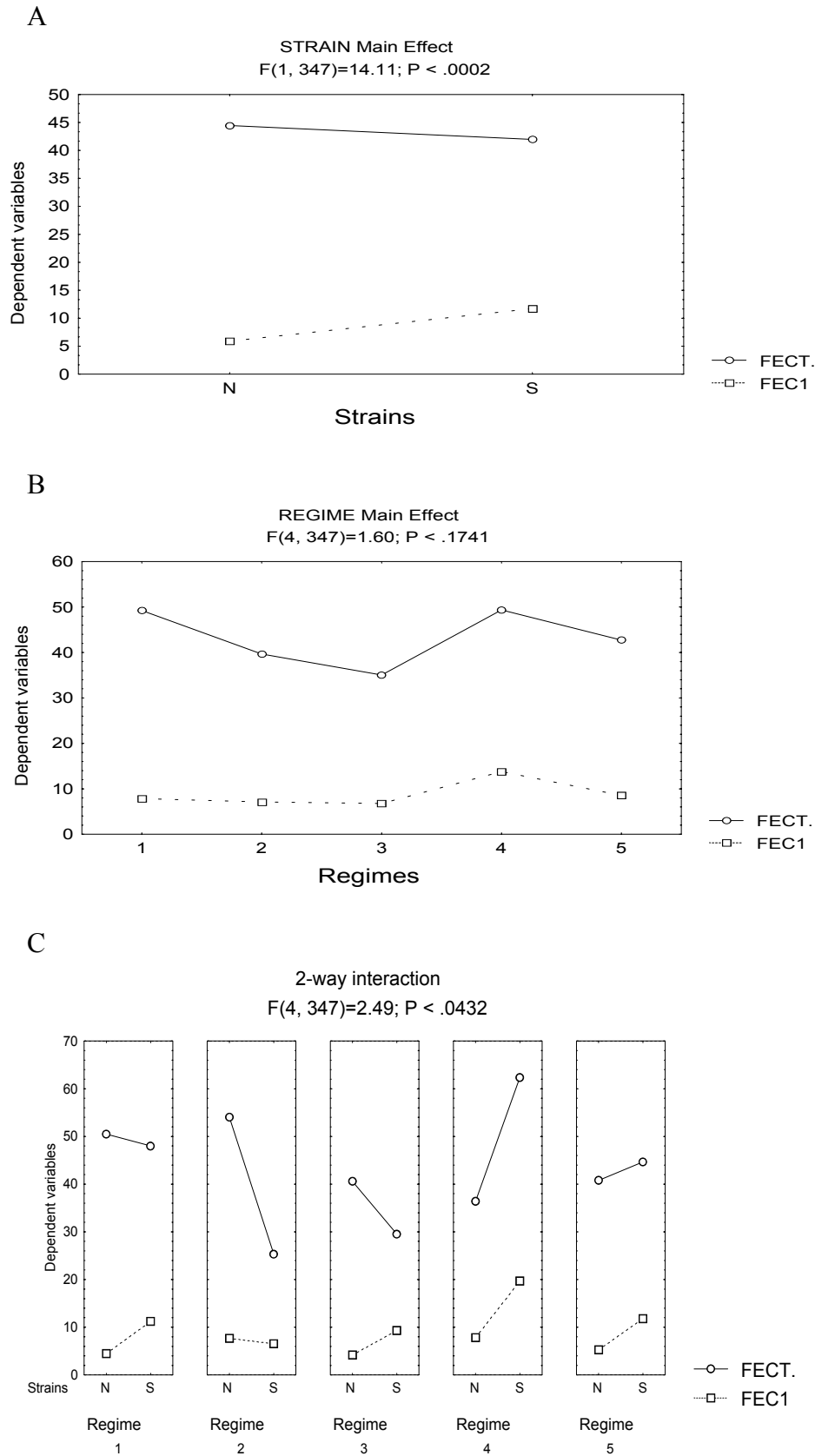
Effect	df	F	P-level	Test of parallelism	
				F	P-level
Strain (1)	1	14.11	0.001 *	16.44	0.001 *
Regimes (2)	4	1.60	0.1	0.94	0.44
Interaction (1 x 2)	4	2.49	0.04 *	2.20	0.02 *

**Table 19.** Number of eggs laid per female by two strains of *O. laevigatus* at the end of exposure 1 and at the end of the experiment 2. Only fertile females were considered. Different letters indicate significant differences found by ANCOVA and Tukey's test ( $P < 0.05$ ) (Means $\pm$ SD).

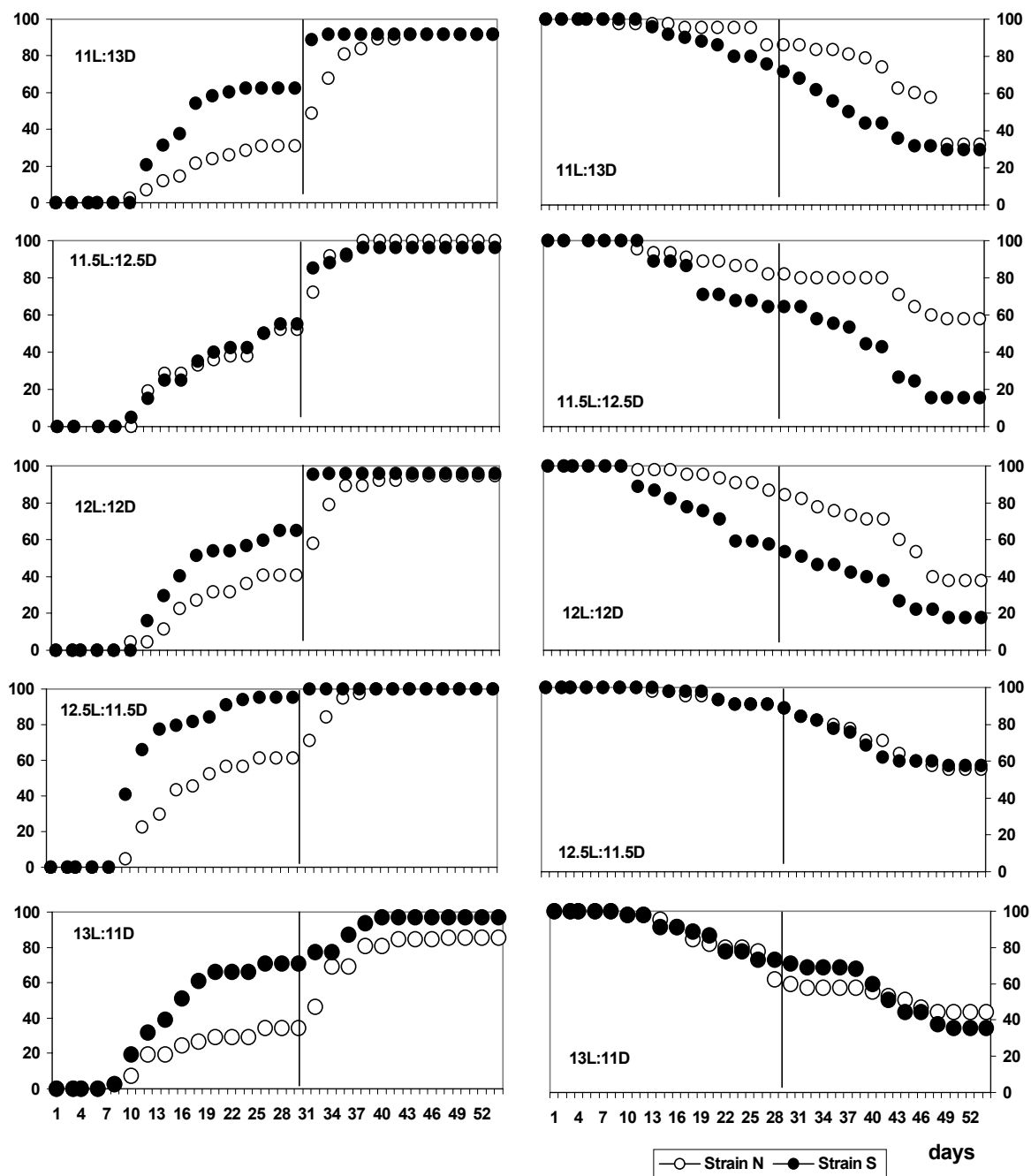
Photoperiod (L:D)	Strain N					Strain S				
	No. of pairs	No. of eggs/fem. Exposure 1	No. of eggs/fem. exposure 1+2	Adjusted means	Fertile females (%) exposure 1+2	No. of pairs	No. of eggs/fem. exposure 1	No. of eggs/fem. exposure 1+2	Adjusted means	Fertile females (%) exposure 1+2
11:13	35	4.5 $\pm$ 8.1 a	50.5 $\pm$ 30.4 a	58.5	91.9	39	11.2 $\pm$ 11.9 ab	48.0 $\pm$ 41.2 a	43.6	91.7
11.5:12.5	38	7.7 $\pm$ 10.8 a	54.0 $\pm$ 31.0 a	56.1	100	29	6.6 $\pm$ 6.9 a	25.3 $\pm$ 27.1 a	29.5	96.3
12:12	39	4.2 $\pm$ 7.7 a	40.6 $\pm$ 28.2 a	49.1	94.7	27	9.3 $\pm$ 9.5 a	29.5 $\pm$ 34.6 a	28.6	95.7
12.5:11.5	41	7.8 $\pm$ 10.4 a	36.4 $\pm$ 31.5 a	38.2	100	43	19.7 $\pm$ 15.8 b	62.4 $\pm$ 54.0 a	42.2	100
13:11	30	5.3 $\pm$ 7.8 a	40.8 $\pm$ 40.2 a	47.3	88.5	37	11.8 $\pm$ 12.6 ab	44.6 $\pm$ 42.8 a	39.1	96.8

**Table 20.** Percentages of fertile and infertile females with mature oocytes 24 hours after the end of the experiment 2.

Photoperiod (L:D)	Strain N				Strain S			
	No. of females	% Fertile female with oocytes	% Infertile female with oocytes	% Infertile female without oocytes	No. of females	% Fertile female with oocytes	% Infertile female with oocytes	% Infertile female without oocytes
11:13	40	65.0	25.0	10.0	42	61.9	7.1	0
11.5:12.5	40	55.0	0	5.0	31	51.6	3.2	6.5
12:12	43	58.1	2.3	7.0	31	35.5	3.2	9.7
12.5:11.5	43	55.8	4.6	0	43	83.7	0	0
13:11	35	54.3	5.7	8.6	37	48.6	0	0
$\chi^2$		P>0.05	P>0.05	P>0.05		P<0.001 *	P>0.05	P<0.05 *



**Figure 6.** Main effects of strain (A), photoperiod (B) and their interaction (C), found in experiment 2 in the covariance analysis (ANCOVA, table 17). (N = northern strain; S = southern strain) (FECT total fecundity, FEC1 the fecundity during exposure 1 at different photoperiods used as covariate) (Regimes: 1 = 11L:13D; 2 = 11.5L:12.5D; 3 = 12L:12D; 4 = 12.5L:11.5D; 5 = 13L:11D).



**Figure 7.** Cumulative percentages of egg-laying (on the left) and surviving (on the right) females of two strains (S=southern and N=northern) of *O. laevigatus* reared at different photoperiods and 18°C for 29 days and then for further 24 days at 16L:8D and 26°C (Experiment 2).

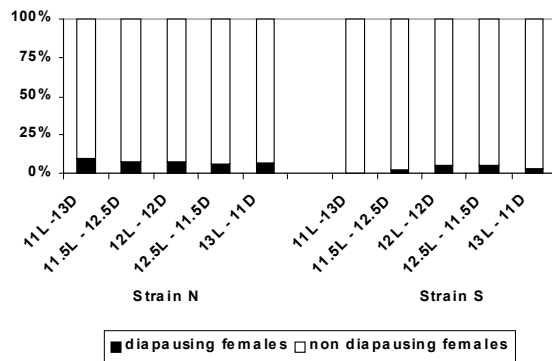
The adjusted means of the oviposition recorded during exposure 2 (when the environmental conditions were switched from 18°C and different photoperiods to 26°C and 16L:8D) show an increase compared to exposure 1 (see figure 7). The highest increase is found for strain N exposed to the photoperiods 11L:13D and 11.5L:12.5D (table 19).

When we compare fertile females with oocytes with infertile females with oocytes and with infertile females

without oocytes for both strains, differences in the percentages of fertile females with oocytes and infertile females without oocytes for the various photoperiods were recorded only for strain S ( $\chi^2$  test,  $P < 0.05$ ) (table 20).

Absence of oocytes in the fertile females could be due to their old age at the end of the experiment, but absence of oocytes in the infertile females could be the result of diapause induction. In any case, only at the photoperiods 11.5L:12.5D and 12L:12D a few infertile females were

found without oocytes, two and three respectively. After exposition for three weeks at 26°C and 16L:8D (exposure 2), none of the females seemed to show diapause in strain S and only a very small number in strain N (table 20). At the end of the experiment a very low percentage of females for both strains appeared to be in diapause for each photoperiod, based on the percentages of egg-laying and non-egg-laying females with oocytes (figure 8).



**Figure 8.** Percentage of egg-laying (= non-diapausing) females and non-egg-laying (= diapausing) females with oocytes at the end of the experiment 2, at different Light – Dark periods.

The cumulative percentages of egg-laying and surviving females during experiment 2 are shown in figure 7. Different effects of photoperiods were found on the percentage of surviving females for both strains at the end of exposure 1 (29th day) (table 21) ( $\chi^2$  test,  $P < 0.01$ ). At the end of the experiment (exposure 1 and 2) a significant difference in egg-laying and survival was re-

corded only for strain S ( $\chi^2$  test,  $P < 0.01$ ). Furthermore, a difference in egg-laying and survival was found between the strains at photoperiod 12L:12D at the end of exposure 1, and at photoperiod 11.5L:12.5D at the end of the experiment 2 ( $\chi^2$  test,  $P < 0.01$ ) (table 21). So, photoperiod can influence female survival.

Total fecundity (exposure 1+2) and the number of mature oocytes per female at the end of the experiment appeared to be weakly correlated ( $y = 39.97 + x$ ;  $R=0.13$ ,  $P < 0.05$ ).

## Influence of temperature

### Experiment 3

This experiment was carried out to study the influence of temperature combined with a critical photoperiod (11.5L:12.5D) on diapause induction of *O. laevigatus*. The ANOVA test showed differences among the regimes of temperatures, but no differences were recorded between strains or their interaction with the regimes on the development time of *O. laevigatus* (tables 22 and 23). The slowest embryonic development and longest development time were recorded for both *O. laevigatus* strains at a thermoperiod 21.5°C/6°C, while the fastest development was at 26°C/15°C (table 23). Furthermore, the development time is shorter at a thermoperiod of 24°C/12.5°C with an average temperature of 18°C than at a constant temperature of 18°C. The pre-imaginal mortality was high at all the regimes tested because groups of ten nymphs were reared together in a cylinder and mortality may be a result of cannibalism. The highest mortality was recorded at 21.5°C/6°C for strain S, while the lowest mortality was observed for both strains at 26°C/15°C ( $\chi^2$  test,  $P < 0.05$ ) (table 24). No differences were recorded for the sex ratio ( $\chi^2$  test,  $P > 0.05$ ), thus all the individuals were considered together for the calculation of the development time.

**Table 21.**  $\chi^2$  test on the percentages of surviving females at various photoperiods and of the two strains at the end of exposure 1 and exposure 2.

Strain	$\chi^2$ test (P-level) at the end of exposure 1	$\chi^2$ test (P-level) at the end of exposure 2
N	$P < 0.005$ *	$P > 0.05$
S	$P < 0.01$ *	$P < 0.001$ *
Photoperiod	(P-level)	(P-level)
11L:13D	$P > 0.05$	$P > 0.05$
11.5L:12.5D	$P > 0.05$	$P < 0.001$ *
12L:12D	$P < 0.01$ *	$P > 0.05$
12.5L:11.5D	$P > 0.05$	$P > 0.05$
13L:11D	$P > 0.05$	$P > 0.05$

**Table 22.** Summary of the main effects by ANOVA on the development time of *O. laevigatus* (strains N and S) during experiment 3.

Effect		Embryonic period			Total development	
		df	F	P-level	F	P-level
Strain	(1)	1	0.44	0.52	4.01	0.07
Regimes	(2)	4	45.49	0.001 *	15.28	0.001 *
Interaction	(1 x 2)	4	0.23	0.92	1.06	0.41

**Table 23.** Pooled means ( $\pm$  SD) of embryonic development time and total development time, considering together the strains N and S of *O. laevigatus*. Different letters on the same line indicate significant differences using ANOVA ( $P < 0.001$ ) and Tukey's test ( $P < 0.05$ ) (Experiment 3).

Temperature regime ( $^{\circ}\text{C}$ )	24/12.5	26/15	21.5/6	22/12.5	18 constant
Embryonic development	$10.0 \pm 0.0$ b	$8.0 \pm 0.0$ a	$12.3 \pm 0.4$ c	$10.6 \pm 1.0$ b	$10.0 \pm 0.0$ b
Total development time	$32.6 \pm 0.7$ b	$28.3 \pm 0.9$ a	$51.3 \pm 1.7$ d	$35.0 \pm 2.2$ c	$36.5 \pm 1.5$ c

**Table 24.** Embryonic development time and total development time (days  $\pm$  SD) of two strains of *O. laevigatus* reared at five temperature regimes matched to the 11.5L:12.5D photoperiod (experiment 3). More than 500 eggs were observed per strain and temperature regime. Pre-imaginal mortality and sex ratio are shown as percentages ( $\chi^2$  test,  $P < 0.05$  for mortality;  $P > 0.05$  for sex ratio).

Temperature regime ( $^{\circ}\text{C}$ )	Strain N				Strain S			
	Embryonic development (days)	Total development (days)	% Pre-imaginal mortality	Sex ratio (% fem.)	Embryonic development (days)	Total development (days)	% Pre-imaginal mortality	Sex ratio (% fem.)
24/12.5	$10.0 \pm 0.0$	$32.4 \pm 0.5$	71.2	47.0	$10.0 \pm 0.0$	$32.7 \pm 0.8$	79.7	54.0
26/15	$8.0 \pm 0.0$	$27.0 \pm 0.0$	68.9	47.0	$8.0 \pm 0.0$	$29.6 \pm 1.7$	48.9	53.8
21.5/6	$12.0 \pm 0.0$	$52.0 \pm 0.8$	83.3	47.3	$12.5 \pm 0.7$	$50.5 \pm 2.6$	88.6	54.8
22/12.5	$10.5 \pm 0.7$	$35.5 \pm 1.3$	83.7	52.3	$10.7 \pm 1.2$	$34.5 \pm 3.1$	83.9	46.2
18 const.	$10.0 \pm 0.0$	$35.9 \pm 2.0$	82.3	45.9	$10.0 \pm 0.0$	$37.0 \pm 0.9$	82.5	47.1

**Table 25.** Summary of the main effects found by ANOVA on the pre-oviposition period and fecundity of *O. laevigatus* (strains N and S) during the exposure at five temperature regimes and 11.5L:12.5D (Experiment 3).

Effect		Pre-oviposition			Fecundity	
		df	F	P-level	F	P-level
Strain	(1)	1	7.42	0.05 *	72.16	0.001 *
Regimes	(2)	4	19.67	0.001 *	22.89	0.001 *
Interaction	(1 x 2)	4	1.71	0.15	6.97	0.001 *

**Table 26.** Pooled means ( $\pm$  SD) of pre-oviposition periods of all the temperature regimes to which the two strains of *O. laevigatus* were exposed. Different letters indicate significant difference by ANOVA ( $P < 0.05$ ) (Experiment 3).

	Strain N	Strain S
Pre-oviposition period (days)	$13.3 \pm 5.8$ b	$11.4 \pm 5.1$ a

The ANOVA (table 25) indicated that strain N had a pre-oviposition period longer than strain S (table 26). Differences were recorded also among the temperature regimes, although the interaction between strains and regimes was not significant. The longest pre-oviposition period was recorded for both strains at 18 $^{\circ}\text{C}$  constant, but this was not significantly different from the data recorded at 21.5 $^{\circ}\text{C}$ /6 $^{\circ}\text{C}$  and at 22 $^{\circ}\text{C}$ /12.5 $^{\circ}\text{C}$  (tables 27 and 28).

Strain S showed a higher percentage of egg-laying females than strain N, particularly at 24 $^{\circ}\text{C}$ /12.5 $^{\circ}\text{C}$ , 26 $^{\circ}\text{C}$ /15 $^{\circ}\text{C}$  and 22 $^{\circ}\text{C}$ /12.5 $^{\circ}\text{C}$  ( $\chi^2$  test,  $P < 0.05$ ) (table 28). When environmental conditions were changed to 26 $^{\circ}\text{C}$  and 16L:8D (exposure 2), sudden increases in the percentage of egg-laying females as well as in the number of eggs laid per female were recorded (figure 9 and tables 31, 32). No differences were recorded for the percentage of fertile females at the end of the experiment, between the strains and temperature regimes ( $\chi^2$  test,  $P > 0.05$ ).

During the initial 29 days of adult life (exposure 1) strain and temperature as well as their interaction seem to have a strong influence on the fecundity of *O. laevigatus* (table 25). The S strain showed a higher fecundity compared to the N strain. For both strains the temperature regime least suitable for oviposition appears to be 21.5 $^{\circ}\text{C}$ /6 $^{\circ}\text{C}$  and 18 $^{\circ}\text{C}$  constant. No differences for the fecundity were observed at 24 $^{\circ}\text{C}$ /12.5 $^{\circ}\text{C}$  and 22 $^{\circ}\text{C}$ /12.5 $^{\circ}\text{C}$  for strain N. The highest fecundity for both strains was recorded at 6 $^{\circ}\text{C}$ /15 $^{\circ}\text{C}$  (table 31).

**Table 27.** Pooled means ( $\pm$  SD) of pre-oviposition periods of all data of the strains N and S of *O. laevigatus*. Different letters indicate significant differences by ANOVA and Tukey's test ( $P < 0.05$ ) (Experiment 3).

Temperature regime ( $^{\circ}\text{C}$ )	24/12.5	26/15	21.5/6	22/12.5	18 constant
Pre-oviposition development (days)	$8.6 \pm 4.4$ a	$9.1 \pm 4.9$ a	$15.1 \pm 5.4$ b	$13.1 \pm 6.2$ b	$15.7 \pm 6.4$ b



**Table 28.** Pre-oviposition period and percentage of fertile females at 29 days after adult emergence ( $\chi^2$  test,  $P < 0.05$ ) of *O. laevigatus* (strains N and S) reared at five temperature regimes and 11.5L:12.5D.

Temperature regime (°C)	Strain N			Strain S		
	No. of Fertile females	Pre-oviposition period (days)	% Fertile females	No. of fertile females	Pre-oviposition period (days)	% Fertile females
24/12.5	21	8.6 ± 4.7	30.6	49	8.5 ± 4.0	96.1
26/15	31	11.4 ± 6.4	64.6	41	6.8 ± 3.4	85.4
21.5/6	17	15.4 ± 5.2	39.5	17	14.8 ± 5.5	43.6
22/12.5	17	14.5 ± 6.6	35.4	38	11.7 ± 5.8	80.8
18 constant	18	16.4 ± 6.0	39.1	28	15.0 ± 6.8	58.3

**Table 29.** Summary of the main effects found by ANCOVA on the fecundity of *O. laevigatus* (strain N and S) considering the fecundity during exposure 1 as covariate. All populations were included (Experiment 3).

				Test of parallelism	
Effect	df	F	P-level	F	P-level
Strain (1)	1	6.40	0.01 *	4.24	0.04 *
Regimes (2)	4	6.92	0.001 *	1.16	0.33
Interaction (1 x 2)	4	1.09	0.36	1.25	0.27

**Table 30.** Summary of the main effects found by ANCOVA and test of parallelism on the fecundity of *O. laevigatus* (strains N and S) considering fecundity during exposure 1 as covariate; only fertile females were included (Experiment 3).

				Test of parallelism	
Effect	df	F	P-level	F	P-level
Strain (1)	1	3.24	0.07	5.74	0.02 *
Regimes (2)	4	8.08	0.001 *	1.60	0.17
Interaction (1 x 2)	4	1.49	0.21	2.05	0.03 *

**Table 31.** Number of eggs laid per female at the end of exposure 1, and at the end of the experiment of the two strains of *O. laevigatus*. All populations were considered. Different letters in column with the same description indicate significant difference by ANOVA and Tukey's test ( $P < 0.05$ ) (Means ± SD) (Experiment 3).

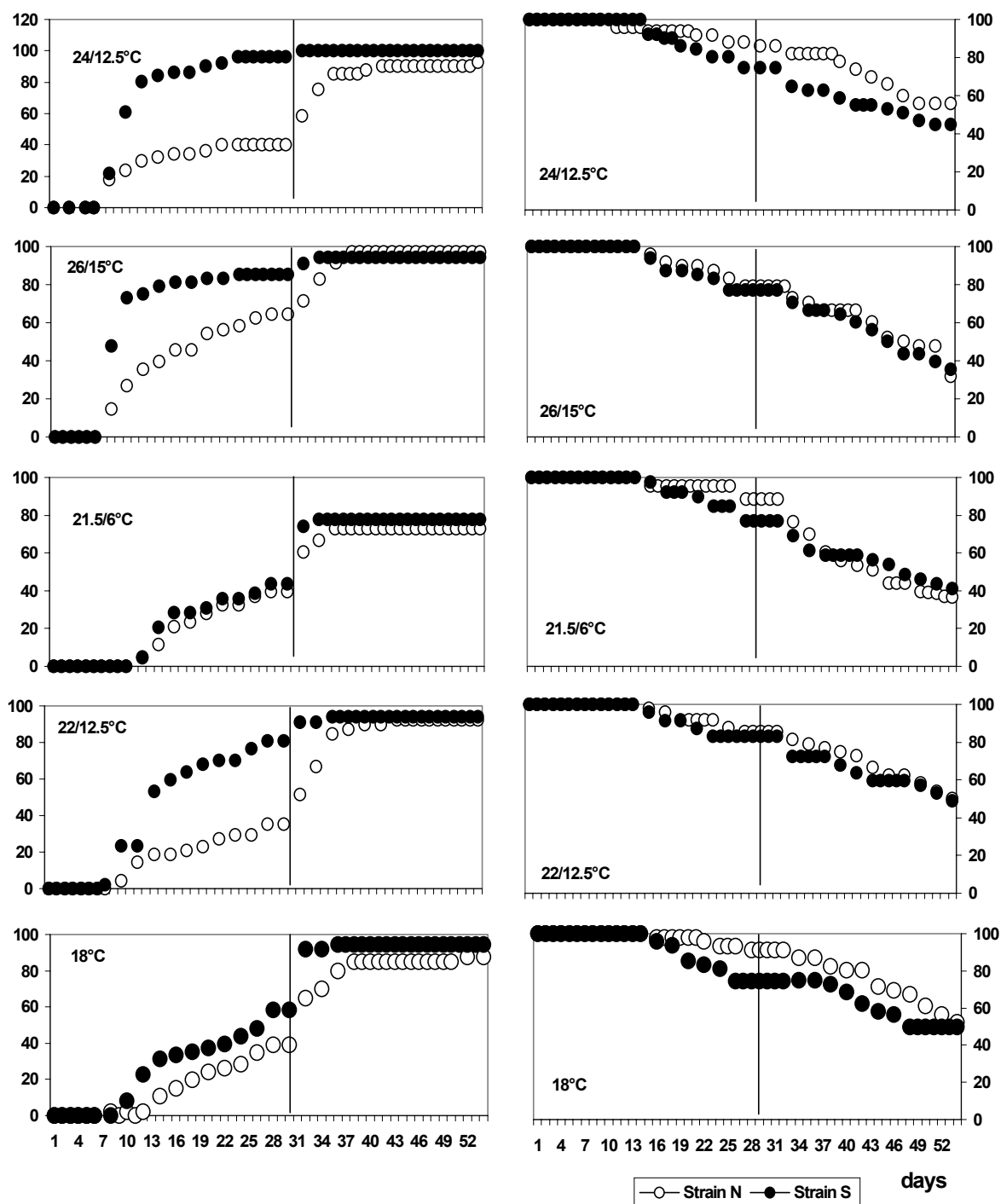
Temperature regime (°C)	Strain N			Strain S		
	No. of pairs	No. of eggs/female exposure 1	No. of eggs/female exposure 1+2	No. of pairs	No. of eggs/female exposure 1	No. of eggs/female exposure 1+2
24/12.5	50	7.2 ± 15.0 a	58.8 ± 58.2 b	51	30.4 ± 20.9 b	76.7 ± 64.4 b
26/15	42	17.7 ± 25.6 b	54.5 ± 57.4 b	39	45.7 ± 30.9 c	92.5 ± 74.6 b
21.5/6	38	5.4 ± 9.1 a	43.4 ± 48.4 a	39	7.8 ± 14.1 a	47.5 ± 57.5 a
22/12.5	48	7.9 ± 14.7 a	65.4 ± 51.5 b	46	24.5 ± 23.1 b	85.7 ± 68.4 b
18 constant	45	5.3 ± 10.1 a	60.6 ± 55.0 b	47	11.6 ± 14.5 a	68.3 ± 57.7 b

**Table 32.** Number of eggs laid per female at the end of exposure 1, and at the end of the experiment of the two strains of *O. laevigatus*. Only the fertile females were considered. The different letters given in the columns indicate significant differences by ANCOVA and Tukey's test ( $P < 0.05$ ) (Means ± SD). Percentages of egg-laying females at the end of the experiment ( $\chi^2$  test,  $P > 0.05$ ) (Experiment 3).

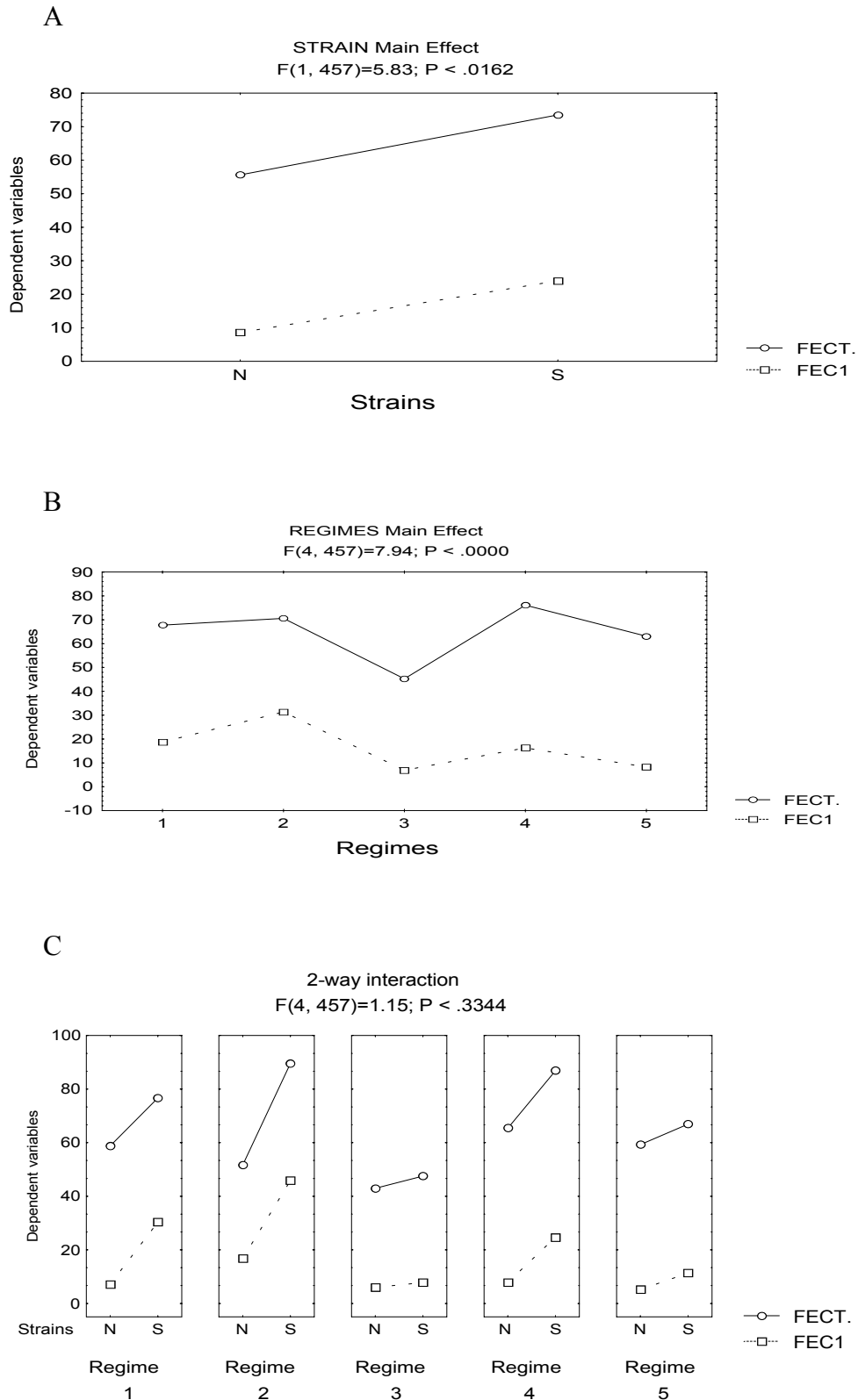
Temperature regime (°C)	Strain N					Strain S				
	No. of pairs	No. of eggs/female exposure 1	No. of eggs/female exposure 1+2	Adjusted means	% Fertile females	No. of pairs	No. of eggs/female exposure 1	No. of eggs/female exposure 1+2	Adjusted means	% Fertile females
24/12.5	37	8.8 ± 16.2 a	71.7 ± 56.6 ab	88.5	74.0	49	31.6 ± 20.4 b	79.8 ± 63.8 b	59.2	96.1
26/15	37	19.2 ± 25.7 b	59.0 ± 56.8 a	58.7	87.5	35	51.2 ± 27.3 c	100.0 ± 66.6 b	47.2	89.6
21.5/6	26	8.9 ± 10.2 a	63.7 ± 48.3 ab	80.3	67.5	25	12.2 ± 16.2 a	74.1 ± 56.4 b	85.4	64.1
22/12.5	39	9.7 ± 15.8 a	80.5 ± 45.1 b	95.8	81.2	42	27.0 ± 22.6 b	95.0 ± 65.5 b	81.8	91.1
18 constant	36	6.5 ± 10.8 a	73.7 ± 52.1 ab	94.3	80.4	35	15.2 ± 14.9 a	89.2 ± 49.6 b	95.5	75.0

Since the environmental conditions were changed in exposure 2 (26°C, 16L:8D), a covariance analysis on fecundity was carried out and the fecundity during exposure 1 was taken as covariate. A covariance analysis was done considering either all females or only fertile females (tables 29 and 30). Differences were found in both cases among the temperature regimes tested. Only

when all females were compared a difference was observed between strains ( $P = 0.01$ ), although the  $P$ -level was almost significant even when only fertile females were considered ( $P = 0.07$ ). No differences on the interaction of regimes and strains were found. Figure 10 shows the main effects of the ANCOVA undertaken on the entire population of females.



**Figure 9.** Cumulative percentages of egg-laying (on the left) and surviving (on the right) females of two strains of *O. laevigatus* reared at five temperature regimes and matched photoperiod (11.5L:12.5D; exposure 1,  $\chi^2$  test,  $P < 0.05$ ) and transferred to 26°C (16L:8D) after 29 days (exposure 2,  $\chi^2$  test,  $P > 0.05$ ) (Experiment 3).



**Figure 10.** Main effects of strain (A), thermoperiod (B) and their interaction (C), found in experiment 3 in the co-variance analysis (ANCOVA, table 29). (N = northern strain, S = southern strain) (FECT total fecundity, FEC1 fecundity during exposure 1 at different thermoperiods used as covariate) (Regimes: 1 = 24/12.5°C; 2 = 26/15°C; 3 = 21.5/6°C; 4 = 22/12.5°C; 5 = 18°C constant).

**Table 33.** Pooled means ( $\pm$  SD) of total fecundity (exposure 1 + 2) considering all the temperature regimes to which the two strains of *O. laevigatus* were exposed. The full populations were considered. Different letters indicate significant differences by ANCOVA ( $P < 0.01$ ) (Experiment 3).

	Strain N	Strain S
Total fecundity (exposure 1+2)	45.7 $\pm$ 54.1 a	74.1 $\pm$ 64.5 b

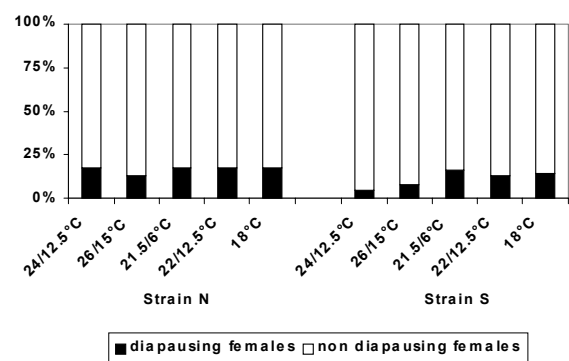
The lowest fecundity was found for *O. laevigatus* strains N previously reared at 26°C/15°C and then transferred to 26°C (exposure 2). It was, however, not significantly different from that of the females of the same strain reared before at 18°C constant, at 24°C/12.5°C and at 21.5°C/6°C (table 32). The adjusted means showed that the smallest effects of a change in exposition were recorded respectively at the temperature regimes 26°C/15°C for strain N and 26°C/15°C and 24°C/12.5°C for strain S. This is the effect of a higher fecundity during the first exposition, which is confirmed by the test of parallelism that correlates the trend of the total fecundity with the covariate (fecundity at exposure 1) (table 29). The total fecundity of strain S at the end of the experiment (exposure 1+2) is significantly higher than that of strain N (table 33). The thermoperiod of 21.5°C/6°C induced the females to lay the lowest amount of eggs (table 34).

At the end of the experiment, no differences among the percentages of fertile females and the infertile females (both groups only with mature oocytes) were recorded ( $\chi^2$  test,  $P > 0.05$ ), while a significant difference was recorded among the percentages of infertile females

without mature oocytes of strain S (table 35). The percentages of diapausing females at the end of experiment 3 were very low (figure 11).

The percentages of egg-laying and of surviving females are shown in figure 9. At the end of the experiment, the percentages of surviving females are similar for both strains at all temperature regimes. No differences in the percentages of surviving females were recorded either at the end of exposure 1 or at the end of exposure 2 between strains as well as among temperature regimes ( $\chi^2$  test,  $P > 0.05$ ).

The correlation between fecundity and mature oocytes per females was significant ( $P < 0.001$ ), but weak ( $R = 0.44$ ). All females were considered together, independently from strain and the exposure ( $y = 43.6 + 6.7x$ ).



**Figure 11.** Percentages of egg-laying (= non-diapausing) females and non-egg-laying (= diapausing) females with oocytes at the end of the experiment (Experiment 3), at different Temperature regimes.

**Table 34.** Pooled means ( $\pm$  SD) of total fecundity (exposure 1 + 2) of all data of strains N and S of *O. laevigatus*. Different letters indicate significant differences by ANCOVA ( $P < 0.05$ ) (Experiment 3).

Temperature regime (°C)	24/12.5	26/15	21.5/6	22/12.5	18 constant
Total fecundity (exposure 1+2)	67.7 $\pm$ 61.3 b	73.5 $\pm$ 66.0 b	45.4 $\pm$ 52.9 a	75.5 $\pm$ 59.9 b	64.4 $\pm$ 56.3 b

**Table 35.** Percentages of fertile and infertile females with mature oocytes 24 hours after the end of the experiment 3 ( $\chi^2$  test) (Experiment 3).

Temperature regime (°C)	Strain N				Strain S			
	No. of females	% Fertile female with oocytes	% Infertile female with oocytes	% Infertile female without oocytes	No. of females	% Fertile female with oocytes	% Infertile female with oocytes	% Infertile female without oocytes
24/12.5	48	56.3	4.2	18.8	51	52.9	0	3.9
26/15	48	56.3	4.2	8.3	48	60.4	0	10.4
21.5/6	42	47.6	2.4	28.6	38	34.2	2.6	31.6
22/12.5	48	70.8	0	18.8	47	48.9	0	8.5
18 constant	45	60.0	0	17.8	47	61.7	4.3	19.1
$\chi^2$		P>0.05	P>0.05	P>0.05		P>0.05	P>0.05	P<0.005 *

**Table 36.** Cumulative percentage of egg-laying females of two strains of *O. laevigatus* (N = strain N and S = strain S) collected in nature in Italy and checked monthly at different intervals in the laboratory (26°C; 75% RH; 16L:8D) (Experiment 4).

Strain	Test period	No. of females	Cumulative percentage of egg-laying females after a certain number of days from isolation at 26°C and 16L:8D				
			day 3	day 7	day 10	day 12	day 21 (total %)
N	August	9	77.8	77.8	77.8	77.8	77.8
N	September	11	100	100	100	100	100
N	October	20	45.0	60.0	65.0	70.0	70.0
N	November	38	2.6	39.5	42.1	47.4	47.4
N	December	18	16.7	55.6	61.1	61.1	61.1
N	January	13	38.5	84.6	92.3	100	100
	$\chi^2$ test		P<0.001	P<0.001	P<0.001	P<0.001	P<0.005
S	August	20	100	100	100	100	100
S	October	39	87.2	89.7	89.7	89.7	89.7
S	November	33	42.4	72.7	78.8	78.8	78.8
S	December	46	41.3	82.6	84.8	84.8	84.8
S	January	55	58.2	89.1	90.9	90.9	90.9
S	February	62	80.6	100	100	100	100
	$\chi^2$ test		P<0.001	P<0.001	P<0.01	P<0.01	P<0.01

#### Incidence of diapause in field collected populations Experiment 4

Adult females collected in nature and kept in a meteorological cabin continued to lay eggs up to mid-November. After that the mean daily temperature decreased to ca. 7.5°C (range 0°C-12.8°C) and many females stopped laying eggs. Some egg-laying occurred again in February when the temperature was around 10°C (range 0°C-17.6°C).

At all times, high percentages of egg-laying females were obtained within a few days after moving them from low temperature regime to optimal environmental conditions. Only in November, strain N did not exceed 48% egg-laying females within 21 days (table 36). When females were transferred from the field to the laboratory during experiment 4, differences in the percentage of egg-laying females of both strains of *O. laevigatus* were recorded ( $\chi^2$  test;  $P < 0.001$  and  $P < 0.01$ ) (table 36). The lowest percentages egg-laying females were observed during the period October-December for strain N and November-December for strain S.

## Discussion

Tauber *et al.* (1986) report that the successful entry into dormancy is determined by the insect's ability to reach the stage sensitive to diapause - inducing - stimuli at the appropriate time of the year. If it reaches that stage too soon, diapause will not be induced and an entire life cycle must take place before diapause can be induced. By contrast, if development is too slow, the insect may not reach the diapausing stage soon enough to avoid the effects of unfavourable conditions.

Because all the *Orius* species known to undergo diapause show a reproductive diapause, the criteria used to diagnose diapausing or non-diapausing females in the present experiments were the same as those used by other

authors (Ali and Ewies, 1977; Ruberson *et al.*, 1991; van den Meiracker, 1994). This means that if females did not start to lay eggs within 29 days after emergence, or if females did not have mature oocytes at the end of the experiment, they were considered in diapause.

#### Influence of photoperiod

##### Development time

For insects which are sensitive to the photoperiod, the 'critical photoperiod' is defined as the length of the day at which 50% of the sensitive stages of the insect will enter diapause (Tauber *et al.*, 1986; Danks, 1987). The critical photoperiod varies from species to species as well as within the same species for strains occurring at different geographical areas. Some insects perceive the photoperiod just as long or short-day (all or none) (Tauber and Tauber, 1976; Tauber *et al.*, 1986). Therefore, the variations in day-length below or above the critical photoperiod are not appreciated by some arthropods, as recorded for example for *Panonychus ulmi* (Koch) (Lees, 1953) and for *Wyeomyia smithii* (Coquillett) (Smith and Brust, 1971; Tauber and Tauber, 1975). The contrary was observed for other arthropods such as *Chrysopa harrisii* Fitch (Tauber and Tauber, 1974) and *Adalia bipunctata* (L.) (Obrycki *et al.*, 1983). Their reaction to a critical photoperiod decreases as diapause progresses. These examples show the limits of the concept of critical photoperiod, because for some insects not only the critical photoperiod but also the gradual change in day-length can influence diapause induction and termination as observed for *C. carnea* (Tauber and Tauber, 1972; 1973; Tauber *et al.*, 1986).

The influence of photoperiod on the development time and adult lifespan of insects was studied by many authors (*e.g.* Danilevskii, 1961; 1970; De Wilde, 1962; Tauber and Tauber, 1976; 1978; Beck, 1980; Saunders, 1982; Principi, 1992). Insects can use photoperiods to regulate their pre-diapause developmental rates, and to

keep growth and development synchronous with the progression of the seasons (Tauber *et al.*, 1986). In many examples, a slow down in development occurs during the actively feeding pre-diapause stages. At the same time, accelerated feeding rates were observed resulting in storage of energy. In some species, short-day-lengths accelerate development and long-day-lengths decelerate it, but in other species it is short-day-length which causes a deceleration (Tauber *et al.*, 1986).

In the studies described in this paper, the preimaginal development time of *O. laevigatus* was shortened by short day-lengths compared to that occurring at long day-lengths. A similar relationship for preimaginal development has been observed for *O. insidiosus* (Ruberson *et al.*, 1991), but it is not always the same among Hemiptera and for *Orius* species (van den Meiracker, 1994).

*O. laevigatus*, as well as other *Orius* species (Ruberson *et al.*, 1991) and many other insects which enter reproductive diapause during winter, show a long-day photoperiodic response curve.

Under each experimental condition, a low percentage of *O. laevigatus* was able to complete pre-imaginal development, and the differences recorded indicate that photoperiod can influence both strains of *O. laevigatus*. In experiment 1, the longest developmental times were recorded mainly around the photoperiod 12L:12D for both strains, while the short-day photoperiod of 8L:16D induced a faster development in both strains. At a long-day photoperiod of 16L:8D, the two strains showed an opposite response: deceleration of development for strain S and acceleration for strain N. When short day-lengths were compared in experiment 2, the intermediate photoperiods 11.5L:12.5D and 12L:12D also induced the longest development time in both strains. An acceleration of the development time induced by intermediate day-length compared to long day-length (16L:8D) was recorded for some *Orius* species which undergo hibernation (McGregor and McDonough, 1917) such as *O. insidiosus* at 10L:14D (Ruberson *et al.*, 1991) and *O. tristicolor* at 12L:12D (Askari and Stern, 1972). This accelerating effect of the development time at short day-length was demonstrated for other Hemiptera, e.g. *Pyrrhocoris apterus* (L.) (Saunders, 1983), *Palomena angulosa* Motschulsky (Hori, 1986; 1987; 1988), *Eysarcoris lewisi* Distant (Hori and Inamura, 1991) and *Podisus maculiventris* Say (Chloridis *et al.*, 1997). However, van den Meiracker (1994) did not find a strict relationship between photoperiod and development time in the species *O. insidiosus* and *O. majusculus*. Also, there are examples of diapausing insects which show a deceleration of the development time at short day-length as *Nezara viridula* L. (Ali and Ewiess, 1977) and *Harmonia axyridis* (Pallas) (Ongagna and Ipert, 1994). For *O. laevigatus*, as well as for *O. insidiosus* (Ruberson *et al.*, 1991), photoperiod induced changes in development time may not necessarily be symptomatic of diapause, but rather may be used to prepare the insect for diapause. *O. laevigatus* shows an opposite behaviour which is very different from other *Orius* species that undergo diapause. At the hypothetical critical photoperiod, this species showed an increase of the development time. Experiments 1 and 2 were carried out at 18°C and this temperature was low

enough to increase the development time and to induce a higher pre-imaginal mortality of *O. laevigatus* (Tommasini, in press; Vacante and Tropea Garzia, 1993).

#### Pre-oviposition and reproduction

The pre-oviposition period is a good indicator for how an adult population reacts to external stimuli and it is often significantly influenced by photoperiod. In fertile females a great variability was recorded, mainly in experiment 1. The longest pre-oviposition periods were recorded at the intermediate photoperiod 11.5L:12.5D for both strains. The variability of the data within the same strain observed during the experiments is similar to the common intra-specific variability which occurs in other insects (Tauber *et al.*, 1986; Danks, 1987).

Photoperiod showed no influence on the oviposition activity of strain S compared to strain N in experiment 1. However, all groups of strains S and N showed a lower percentage of egg-laying females in comparison to strain N reared at 16L:8D. Photoperiod strongly affected oviposition of strain N. A photoperiod 12L:12D induced a reduction in both the number of eggs laid per female and the percentage of egg-laying females, compared with 16L:8D. Experiment 1 showed that a critical photoperiod for diapause induction could be around 12L:12D hours. This induced a long development time in both *O. laevigatus* strains and a low oviposition rate in strain N. In test 1 of experiment 1, only 31.7% of females of the strain N laid eggs and in test 2 of the same experiment only one female of the same strain laid eggs after 23 days of adult life. The low percentages of egg-laying females in strain S (all day-lengths) and in strain N (short and intermediate day-lengths) could indicate a high incidence of reproductive diapause, according to the criteria of van den Meiracker (1994). The lack of a photoperiodical response in the oviposition activity of strain S suggests, however, that in Sicily or at lower latitudes, *O. laevigatus* may overwinter in quiescence, or that only a low percentage of the population undergoes diapause.

Furthermore, a low temperature such as 18°C reduces the oviposition capability of *O. laevigatus* like in other *Orius* species (Alauzet *et al.*, 1994; Tommasini and Benuzzi, 1996) even at a long photoperiod. This decreased oviposition does not mean that diapause induction has occurred. Short day-lengths between 8L:16D and 12L:12D strongly influenced strain N to initiate diapause, but not in strain S.

Therefore, to study the existence of diapause in strain S, further studies were needed. These studies concerned the influence of photoperiods around 12L:12D. The effect of the increase of temperature after a period at 18±1°C (pre-imaginal development and part of adult life) were tested also for *O. majusculus* and *O. insidiosus* (van den Meiracker, 1994). In experiment 2, the critical photoperiod for strains S appeared to be between 11.5L:12.5D and 12L:12D, with a longer development time, lower fecundity and mainly lower percentage of egg-laying females during the initial 29 days of adult life. But when the environmental condition were switched to 26°C and 16L:8D, most females (>90%) suddenly started to lay eggs. During adult life, the two

strains of *O. laevigatus* reacted differently to the different photoperiods. In particular the fecundity of strain N seemed to be less influenced by a narrow range of photoperiods compared to that of strain S. In fact, some species of insects living in southern areas (mainly near the tropics) frequently react much more to small changes of photoperiod than the same species living in northern areas, which are exposed to a much larger variation in photoperiod during the year (Beck, 1980). Among insects reared at an intermediate photoperiod, the incidence of diapause was higher when temperature was relatively low than if it was relatively high (Danilevski, 1961; Beck, 1980).

The beginning of oviposition of hibernating females under diapause-promoting photoperiods has been considered to indicate the completion of diapause (Tauber and Tauber, 1976). The length of the pre-oviposition period could be a parameter to study the intensity of reproductive diapause in long-day conditions, as considered by Hodek and Honek (1970) for another heteropteran, *Aelia acuminata* (L.).

It is known that latitude and altitude can have an effect on the photoperiodic response of insects. Saunders (1982) and Danks (1987) report that the critical photoperiod changes to longer values with increasing latitude. The intensity of diapause was found to be greater in northern than in southern populations of several insect species (Beck, 1980).

In experiment 2 differences in fecundity were recorded between the two strains of *O. laevigatus*, but they were not caused by a difference in photoperiods. Only strain N showed differences in the percentage of fertile and infertile females with mature oocytes at the end of the experiment. It appears that for *O. laevigatus* also the geographical origin plays a specific role in the diapause induction or at least on some biological parameters. Several authors made a list of species which showed differences in critical photoperiod between populations from different latitudes (Masaki, 1961; Beck, 1980; Danks, 1987). A low incidence of diapause in more southern populations was observed in several insect species exposed to a short photoperiod (Danks, 1987). Geographical differences in the photoperiodic response at different localities at the same latitude have been investigated for a number of species. Populations of *Laodelphax striatellus* (Fallén) occurring in more northern areas have evolved a geographical strain entering diapause in earlier instars compared to that of more southern regions (Kisimoto, 1989). *Mamestra brassicae* (L.) as well as *C. carnea* showed a shorter critical photoperiod at more northern than more southern latitudes (Danilevskii, 1961; Tauber and Tauber, 1972).

#### Influence of temperature

##### Development time and reproductive period

The interaction between temperature and photoperiod may be important for diapause induction and intensification (Honek, 1969; Beck, 1980; Volkovich *et al.*, 1992). Kingsley and Harrington (1982) as well as van den Mairacker (1994) demonstrated this interaction also

for diapause termination of *O. insidiosus*. Temperature can influence the critical photoperiod, and a high temperature generally prevents diapause induction (Beck, 1980; Danks, 1987; Volkovich *et al.*, 1992). Under natural conditions, insects are exposed to thermoperiods in combination with photoperiods, where night-time temperatures are generally lower than daytime temperatures. For this reason, thermoperiods were used during experiment 3 instead of constant temperature used in the past by many authors to study this phenomenon. A mean temperature of 20°C (thermoperiod 26°C/15°C) combined with a short-day-length, seems enough to prevent diapause in at least 64.6% of females of strain N and in more than 85% of strain S of *O. laevigatus*. This was also found for other insects like *Corythucha cydoniae* (Fitch) where diapause did not occur in at least 25% of the population at high temperatures and short daylength. Neal *et al.* (1992) are of the opinion that a mean temperature of about 20°C combined with a short-day-length can reduce diapause intensity. Thermal modification of photoperiod is rather common in insects.

The influence which the temperature can have on the biology of *O. laevigatus*, (mainly on strain S), is demonstrated by the high pre-imaginal mortality and low percentage of fertile females recorded at the mean temperature regime of 16.5°C. As already discussed, *Orius* spp. are rather sensitive to low temperatures independently from photoperiod. Chyzik *et al.* (1995) studied an Israeli strain of *O. albidipennis* and found that a mean temperature below 15°C leads to an interruption of oviposition irrespective to day-length until the temperature increases. High temperatures can avert diapause in long-day insects. De Wilde (1962) and Masaki (1984) reported a great variation in reaction types of diapause which can occur among different geographic populations of the same species at the same temperature stimuli. Commonly, higher environmental temperatures are required to avert diapause induction among the northern forms than among the southern forms of the same species (Beck, 1980). In fact, strain S of *O. laevigatus* showed to be less sensitive to the different temperature regimes tested with, on average, a shorter pre-oviposition period (9.4 days) than strain N (13.3 days), as well as a higher percentage of egg-laying females. In a variety of species adaptation to low latitude is related more closely to environmental temperature patterns than to day-length effects associated with differences in latitude (Beck, 1980). Low temperature did induce diapause in several species of flies close to the equator, when day-length changed by only a few minutes throughout the year and, therefore, photoperiod was not found to play a role in diapause induction (Saunders, 1982).

In *O. laevigatus* a difference in diapause reaction was recorded for both strains when tested at the temperature of 18°C constant or at thermoperiod 24°C/12.5°C with a mean temperature of 18°C. The constant temperature regime induced a pre-oviposition period almost two times longer than that at the varying temperature regime. A similar result was found by Rudolf *et al.* (1993), who observed reduced longevity and fecundity of both *O. laevigatus* and *O. majusculus* at low constant

temperatures, compared to a thermoperiod with the same average. These results are not in agreement with the theory put forward by Beck (1977), in which the growth-rates produced at the thermoperiods are rather similar to those produced at comparable average temperatures.

The results of the dissection of females at the end of the third experiment made clear that the conclusion stating that females which did not lay eggs were in diapause, was incorrect. These results allow us to conclude (1) that *O. laevigatus* is influenced by short day-length and low temperature regimes to initiate diapause, (2) that only part of the population reacts to these token stimuli by entering diapause, and (3) that the northern strains are more sensitive to these stimuli than the southern strains.

#### Incidence of diapause in field collected populations

Both strains of *O. laevigatus* used in experiment 4 were collected in the field and immediately tested in laboratory. This was done to prevent the possibility of selecting a strain where the sensitivity to a short-day-length is lost under laboratory conditions, like Hodek and Honek (1970) found for *A. acuminata*. When collecting *O. laevigatus*, only a low number of males were found (sex ratio of 1 male to 10 females), but males of *O. laevigatus* commonly live shorter than females. When collecting *O. tricolor* and *O. insidiosus*, males were also rarely found in the field during winter (Anderson, 1962; Iglsinsky and Rainwater, 1950; Kingsley and Harrington, 1982). Frequent mating was observed during our experiments at different environmental conditions. Because mating usually does not occur during diapause (Tauber *et al.*, 1986) an observation of mating could suggest the absence of diapause for at least part of the population of *O. laevigatus*. Although mating was observed also by Ruberson *et al.* (1991) for *O. insidiosus* in the laboratory under short day-length, recent studies failed to recover males of *O. insidiosus* during the late winter and early spring (Elkassabany *et al.*, 1996) and overwintering females appear to be inseminated before winter (Elkassabany, 1994).

Iglsinsky and Rainwater (1950) showed that females of *O. insidiosus* collected during winter in the USA laid only ten eggs during the 45 days they were alive. More recently, Elkassabany *et al.* (1996) demonstrated that all the females of *O. insidiosus* collected in Arkansas (USA) at the beginning of November were in diapause. This confirms Ruberson *et al.*'s (1991) data, since *O. insidiosus* showed a critical photoperiod which corresponded with that of mid-October in the same area. A recent laboratory study carried out in Brasil from Argolo *et al.* (2002) showed that *O. insidiosus* did not enter in reproductive diapause at any of 6 photoperiods with ranged from 9L:15D to 14L:10D, at the constant temperature of 25±1°C and RH 70±10%. This results can be due to the behaviour of a strain of *O. insidiosus* collected in Brasil respect those studied by other authors whom collected the predators in North America.

*O. laevigatus* has a different behaviour compared to that of *O. insidiosus* or other *Orius* species. In particular, the females of strain S of *O. laevigatus* collected in

winter reacted quite suddenly to an increased temperature by starting to lay eggs. A quicker reaction of strain S to begin laying eggs was found during almost the entire winter compared to strain N. The geographical origin of the two strains strongly influenced the start of the oviposition. Throughout autumn and winter, at least 79% of females of strain S started to lay eggs ten days after moving the females from the field (low temperature) to the laboratory (26°C), while in strain N only about 48% of the females moved to 26°C laid eggs in November. This suggests that in nature, diapause is induced in strain N of *O. laevigatus* when day-length and temperature decrease in autumn. The differences in reaction to critical photoperiod between these strains are large enough to propose the existence of two ecotypes of *O. laevigatus*. Further, it seems reasonable to conclude that in both strains of *O. laevigatus*, only part of the population undergoes diapause and an intensive, obligatory diapause seems to lack.

It is not easy to define the overwintering behaviour of *O. laevigatus* with one of the descriptions of diapause given by different authors. However, according to Müller's classification (1970), which is used by many authors in Europe, it is possible to say that the northern strain of *O. laevigatus* shows an oligopause while the southern strain shows only a quiescence form of overwintering. Other authors prefer to describe this oligopause as 'facultative diapause' (Beck, 1980), whereas Tauber *et al.* (1986) point out that for 'non-diapause' overwintering forms the definition 'multivoltine diapause' is more reliable than 'facultative diapause'.

#### Conclusions

The presence or absence of diapause is an important criterion to select a good natural enemy, mainly when the pest can overwinter without undergoing diapause. This is true for both predatory insects and mites. For example, when diapausing strains of *Amblyseius* (= *Neoseiulus*) *cucumeris* (Oudemans) were released in winter or early in spring, they were not successful in controlling WFT (van Houten and van Stratum, 1993).

Diapause appears to be a widespread phenomenon among Heteropteran predators in temperate areas. It has an important function in enhancing survival during adverse seasons and in synchronizing the predators with prey in spring. However, when such predators are used against prey lacking diapause (*i.e.* *F. occidentalis*), the importance of understanding if a species or a strain can synchronize its behaviour with that of the target prey is very important and useful for biological control.

No specific studies were previously carried out on the influence of photoperiod and temperature on the diapause induction of strains of *O. laevigatus*. Only some information was available on a southern Italian strain (Vacante *et al.*, 1995) where photoperiod did not seem to influence fecundity.

One of the main findings of these studies is that a northern (N) and a southern strains (S) of *O. laevigatus* are different. The difference in behaviour observed allows us to distinguish two ecotypes of this species.



Northern strains showed a weak diapause, while the southern population demonstrated a very low incidence of diapause and most of the individuals seem to have only a quiescence form of overwintering. However, also the southern strain of *O. laevigatus* appeared to be strongly influenced by low temperature.

Adults of *O. laevigatus* of both N and S strain, collected in nature in autumn-winter, then kept under field conditions, and eventually transferred after a certain period to controlled and optimal environmental conditions, showed that part of the females of strain N undergo reproductive diapause during November. However, their diapause could be considered rather short, because the percentage of egg-laying females increased a lot just a month later, in December.

The southern strain (strain S) collected on Sicily showed no evident response to the photoperiod, indicating that they can overwinter in quiescence or that their eventual diapause, if any, is weak. Therefore, it seems that strains collected at low latitudes can remain active also during short-day-length periods, if temperature is high enough, confirming the finding of Chambers *et al.* (1993). Moreover, the natural distribution of *O. laevigatus* in areas with marine influence (Péricart, 1972) and the good performances at high temperatures (Alauzet *et al.*, 1994) indirectly explain the effectiveness of this species also during the hot season in both the Mediterranean area and northern Europe.

The intrinsic variation among individuals that we found seems to be a characteristic of many diapause responses in other species (Danks, 1987). Commonly diapause incidence and intensity tend to increase with a higher latitude and altitude and the intrinsic features of the diapause response interact with environmental factors. Often the critical photoperiod is longer and the initiating temperature is lower for northern strains (Danks, 1987).

While an insect's diapause response is genetically based, environmental conditions determine the expression of diapause (Tauber *et al.*, 1986). Other stimuli, *e.g.* food abundance and quality or changing versus static photoperiods, can influence diapause in interaction with photoperiod and temperature (Tauber *et al.*, 1986). Low quality food can shift the critical photoperiod for predatory Heteroptera, but only slightly (Ruberson *et al.*, 1998). Because we did not compare several food resources, we do not know how food influences diapause in *O. laevigatus*. The importance of feeding for diapausing adult predators is unclear, although diapausing Heteropteran predators without prey did not survive better than starved non-diapausing predators. It could be that Heteropteran species must locate overwintering areas where some prey is available (Ruberson *et al.*, 1998).

To correctly describe the diapause of *O. laevigatus* is not an easy task. When we summarise the important elements of diapause given by several authors, it is possible to distinguish two main criteria to define the intermediate form of diapause that we found for *O. laevigatus*:

- presence or absence of a physiological adaptation
- degree of reaction to the environmental conditions that induce and terminate dormancy (diapause).

Interpretation of these criteria results in the conclusion that the N strain of *O. laevigatus* shows a dormancy similar to an 'oligopause' (according to Müller, 1970) or a diapause with a low intensity (according to Mansingh, 1971). For strain S we may speak of a 'quiescence' mainly influenced by temperature.

The applied importance of this diapause study in *O. laevigatus* is the following. For natural enemies used in biological control of thrips during winter and early spring, it is essential that they do not enter diapause. This resulted in a search for non-diapausing natural enemies of thrips for winter and spring when seasonal inoculative releases are necessary. The finding that southern (in this case Sicilian) strains of *O. laevigatus* does not show diapause but quiescence is an important result to improve biological control of thrips.

The southern Italian strain of *O. laevigatus* is currently produced and commercially used on large scale in Europe to control thrips species in vegetable and ornamental crops, mostly in protected crops.

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